Causes and consequences of sexual selection in a wild population

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Submitted for the degree of Doctor of Philosophy

The University of Edinburgh

2011

Declaration

I have composed this thesis. Although the long-term data used in many of the analyses was collected by others, I have contributed to the long–term data collection over the last four years, giving me a full understanding of the methods used. I have undertaken all of the analysis presented, and written the thesis. The only exception to this is that Dr Dan Nussey constructed figure 4.1, and that I have incorporated feedback from co-authors and supervisors. This work has not been submitted for any other degree or professional qualification.

Acknowledgements

First and foremost, I'd like to say how lucky I am to have had such fantastic supervisors in Josephine Pemberton and Dan Nussey. Josephine has been a wonderful source of wisdom and encouragement throughout my PhD, striking the perfect balance between thorough and careful commentary and allowing me the space to develop as a scientist. Dan's passion and excitement about the work I've done, and about science in general, have been incredibly inspiring, and he has combined constructive critique with kindness and generosity. I hope your first student has been a great adventure for you Dan! I'd also like to thank Steve Albon as a CASE supervisor for his helpful input. And of course, thanks to NERC for funding my PhD, and the Macaulay Institute for CASE funding.

Tim Clutton-Brock has made a vital contribution to this thesis in many ways. The effort he has invested in creating and maintaining the Red deer project has resulted in a fantastic resource, and I am so grateful to have had the opportunity to be part of this project. Tim has constantly challenged me to think harder about the questions I am trying to answer, and I'm glad to have the opportunity to debate ideas with such a legend of behavioural ecology. We may not have always agreed, but I've learnt a lot, so thank you. I also owe a huge thanks to all those involved in the collection of field data on the Rum project. In particular, I'd like to thank Fiona Guinness, for her incredible work over five decades. If I am ever half as patient, observant and careful as she is when doing fieldwork, I will be very proud. Ali and Sean Morris have also taught lots about deer and how to do fieldwork properly. They have been really kind to me, as far back as my very first trip to Rum: a big thank you for getting me to that interview! I'm really lucky to have started on Rum at the same time as Martyn Baker. Thanks Martyn for the fun, the trust you put in me and fantastic birthdays. In general, thanks to everyone I've done fieldwork with- Michelle Clements, Hannah Granroth-Wilding, Siobhan Simpson, Andrew Fisher, Chris Dowson and many others- you made it a lot of fun.

There are many, many others who've made a huge difference professionally. Thanks to the whole Wild Evolution Group at Edinburgh for thoughtful insights and ideas. A particular thanks to Jarrod Hadfield, Ian White and Alastair Wilson for statistical help. Thanks to Ian Stevenson for building a fantastic database that *almost* made all the data entry a pleasure... Thanks also to Tim Coulson, Mick Crawley, Jon Slate, Kelly Moyes

and the Large Animal Research Group at Cambridge for useful discussions, particularly over the elusive female choice conundrum.

I'd like to thank my parents, Ted and Joy Stopher, for supporting me and helping me to follow my passions. I've made some incredible friends whilst in Edinburgh. There are too many lovely people to mention, but a particular thanks to those with whom I've shared this adventure with: Laura Pollitt, Anna Auld, Adam Hayward, Claire Bourke, Gethin Evans, Stuart Auld and Ben Longdon. We made it guys! Last, but not least, I'd like to thank Craig Walling. Craig's mentoring has been invaluable, and I've enjoyed the many hours of debate over the finer points of sexual selection. He has also kept me sane, made me smile and been a brilliant partner for our many adventures. Here's to many more...

Abstract

Although sexual selection in nature has been studied intensively, much is still unknown about the evolution of mating systems in wild populations: for example, how male competition and female choice interact, or the effect of environmental heterogeneity on selection. Further, important questions remain about the consequences of sexual selection for genetic structuring and genetic variation within populations. In this thesis, I investigate the causes and consequences of sexual selection in a polygynous mammal, the red deer *Cervus elaphus*. This species is characterized by high male reproductive skew resulting from competition to defend harems of females. Here however, I present evidence for previously unappreciated complexity in the mating system, in terms of female mating behaviour and environmental influences on male-male competition. I then go on to investigate the consequences of non-random mating on co-ancestry and inbreeding in the population. Finally, I investigate methods for separating genetic and environmental sources of covariance between individuals. Specifically, I:

(i) Show a surprising degree of female mobility during the breeding season (the 'rut'). Around 40% of females change harem when in oestrus and almost half of these movements result in paternity for the novel male; however I show that these movements are unlikely to be explained by female choice for mates.

(ii) Reveal that variance in male mating success is affected by variation in ecological parameters, in particular the interaction between the number of immigrant males in the rutting population and the temporal synchrony of females.

(iii) Demonstrate substantial inter-individual differences in the plasticity of acoustic signals produced by rutting males with changes in social context.

(iv) Reveal the existence in this population of three rarely reported mating behaviours in polygynous mammals. I find around a fifth of females mate with the same male in multiple years; female relatives frequently mate with the same male; and males rut in locations close to their relatives. Further, I show these behaviours are assoicated with higher co-ancestry and inbreeding in the population than expected under random mating.

(v) Finally, I investigate how spatial associations between relatives upwardly bias estimates of heritability in four phenotypic traits. I do this by accounting for shared environment effects in animal models by i) inclusion of spatial autocorrelation parameters and ii) a novel multi-matrix approach.

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Chapter 1

General Introduction

1.1 Sexual selection in wild populations

'We are, however, here concerned only with that kind of selection, which I have called sexual selection. This depends on the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction' (Darwin, 1871).

Sexual selection is a powerful evolutionary force in nature, shaping the evolution of elaborate ornaments, weapons and animal communication, determining sex roles and sexual dimorphism, and influencing speciation and population persistence or extinction (Andersson 2004a, Lande 1980). Yet, despite the intense research that has gone into this field in recent decades, there are still many uncertainties about the fundamental principles of sexual selection: why does it occur and why does it vary within and between lineages (Jones and Ratterman 2009)? Added to this, new levels of complexity revealed by both theoretical and empirical studies have raised further questions which have been highlighted in recent reviews. These include the total extent of selection when multiple sexual selection processes occur in a population (Hunt *et al.* 2009); how environmental heterogeneity affects sexual selection (Ingleby *et al.* 2010, Cornwallis and Uller 2009); the role of phenotypic plasticity in shaping sexually selected traits (Cornwallis and Uller 2009); and why directional mate preferences do not appear to erode variation in selected traits (the 'lek paradox', Taylor and Williams 1982, Rowe and Houle 1996).

Laboratory experiments have made, and continue to make, important insights into these and other questions in this field, because they offer clear opportunities to test hypotheses under controlled conditions (for example, see studies reviewed in Andersson 2004a, Hunt *et al.* 2009, Ingleby *et al*. 2010). However, studying wild populations allows us to incorporate real-world variability in the factors affecting selection, in comparison to laboratory conditions which are generally benign, stable and novel and in which selection is therefore likely to differ from natural conditions (Ellegren and Sheldon 2008). Further, the importance of understanding how environmental processes affect evolutionary processes in natural environments is increasingly important as we try to predict how

populations may change in response to anthropogenic climate change (Visser 2008, Kruuk and Hill 2008).

In this thesis, I investigate the causes and consequences of sexual selection in a wild population of red deer. Here, I outline the key concepts of sexual selection (sections 2 and 3) and the causes of variation in sexual selection within and between populations (section 4) and discuss the consequences of this variation for the maintenance of genetic variation and population genetic structure (section 5). I then briefly outline how the population genetic structure can result in spatial heterogeneity in trait distribution and the relevance of this to quantitative genetic studies (section 6). Finally, I describe the study population and how this thesis addresses the theoretical concepts of sexual selection introduced in this chapter.

1.2 What is sexual selection?

The working definition of sexual selection used by most modern researchers differs little from when Darwin first identified it: sexual selection arises when some individuals of one sex are able to monopolize access to mating with the other sex, so that only a subset of individuals pass on their genes (Darwin 1871, Andersson 1994a, Jones and Ratterman 2009). Note however, that such definitions are mainly focussed on pre-copulatory sexual selection, and in some species the importance of post-copulatory selection is now well realised (see Birkhead 2010). Darwin identified two major processes of sexual selection: male competition for access to females, and female choice to mate with preferred males. Male competition, or intrasexual selection, is by far the more obvious of the two, with many widely known examples, such as fights between male red deer, elephant seals or stalk-eyed flies (Clutton-Brock *et al.* 1979, le Boeuf 1994, Panhuis and Wilkinson 1999). However, many studies have also demonstrated female mating preferences for male phenotypic traits such as large body size, vivid colouration, display or song (reviewed in Andersson 1994a) and as long ago as Darwin's research, it was recognized that in species that are characterized by male competition, female choice can still be important. Recently much effort has been devoted to understanding how the two processes interact (see section 1.4.1).

A key unresolved question in sexual selection is why female preferences evolve. Direct benefits to mating with a preferred male present little theoretical challenge and are well supported empirically: these include nuptial gifts (Cordero 1996), parental care (Borries *et al.* 1999, Buchan *et al.* 2003, Little *et al.* 2008), fertility (Preston *et al.* 2001), protection

from harassment (Carborne and Taborsky 1996) and reduced risk of punishment (Manson 1994). However, the evolution of female choice in species where males appear to provide nothing other than their genes invokes the possibility of indirect benefits, of 'good genes' or 'compatible genes'. These remain controversial due to lack of firm empirical evidence, and theoretical difficulties such as the small magnitude of predicted benefits (Kokko *et al.* 2003). In 'good genes' models females are predicted to prefer males who will pass on genes to their offspring which increase offspring viability and/or reproductive success. In contrast, 'compatible genes' models predict females should prefer to mate with males whose genes will produce favourable combinations of genes in the offspring when combined with the genes of the female, and therefore increase offspring viability (Trivers 1972, Mays and Hill 2004). For example, females may prefer to mate with males which are optimally genetically dissimilar to them in order to increase the heterozygosity of their offspring (Amos *et al.* 2001, Hansson & Westerberg 2002).

1.3 Measuring sexual selection

The measurement of sexual selection in natural populations is a challenging area and one under constant debate (Andersson 1994a, Koenig and Albano 1986, Kokko *et al.* 1999, Mills *et al.* 2007a, Jones 2009, Klug *et al.* 2010a). Selection must be distinguished from evolutionary response to selection: selection causes observable changes in phenotypes within generations, and is therefore not dependent upon the heritability of traits, whereas the evolutionary response to selection will depend upon the inheritance of the trait (Arnold and Wade 1984). Selection is therefore the statistical relationship between fitness and phenotypic trait within a generation, and this covariance is known as the selection differential, the change in phenotypic mean after selection compared to before selection (Lush 1945, Falconer 1989, Arnold and Wade 1984). However, it has long been recognised that the observed selection may be either a result of selection on the trait considered or on correlated traits (Lande 1979, Lande and Arnold 1983). If data exists on the correlated traits, the partial regression of relative fitness on a trait can be used to measure selection on that trait; this is known as the selection gradient (Lande 1979, Lande and Arnold 1983).

Whilst selection gradients are probably the best available way to measure sexual selection, doing so is challenging in natural populations because of the difficulties of gathering data on all traits that are potential targets of selection. Even if all traits could be identified, as the number of traits increases the number of individuals needed for analysis increases at

an approximately quadratic rate (Andersson 1994a). However, these methods have been employed by a number of researchers: for example, studies of sexual selection on body size, beak size, territory size and quality and plumage colour in Darwin's finches revealed both direct and correlated selection on these traits (Price 1984, Grant 1985, Grant and Grant 1987, 1989) and Sheldon and Ellegren (1998) used multivariate selection analyses to show positive directional sexual selection on two uncorrelated traits in male collared flycatchers, forehead and wing patch size.

An alternative set of measures of sexual selection do not measure selection *per se* but rather the potential for sexual selection. Such proxies are based on the concept that the variance in relative mating success among males constrains the intensity of selection that can act on any trait, and as such sets the upper limit for selection (Crow 1958, Arnold and Wade 1984). Measures in this suite include the opportunity for sexual selection (a standardized measure of intra-sexual variation in mating success, I_s, Crow 1958), the Bateman gradient (the relationship between mating and reproductive success for a given sex, Arnold and Duvall 1994) and Morisita's Index (the observed variance in mating success corrected for the variance expected when the probability of acquiring a mate is equal, Morisita 1962). These are reviewed in Kokko *et al.* (1999). Whilst such measures are widely employed, care must be taken to remember that such measures can only indicate the upper limit of sexual selection, rather than the true strength of selection. For example, a recent theoretical study has shown that the conditions under which opportunity for sexual selection and selection gradients are correlated are restrictive (Klug *et al.* 2010a).

Further, it has been noticed recently that which individuals are included in measures of sexual selection can affect interpretation of the magnitude of selection. Shuster and Wade (2003), and Wade and Shuster (2004) argue that measures of selection which exclude males that do not mate underestimate the true strength of selection. For example, if males have to obtain a territory before they can obtain a mate, failing to include males who are unsuccessful in this initial competition will underestimate the total strength of sexual selection. Klug *et al.* (2010b) showed that the effects of density and nest limitation on sexual selection in male sand gobies differed depending upon which episode of selection, and therefore which group of males, was considered. This problem is essentially one of distinguishing between episodes of natural and sexual selection. This boundary is controversial (*e.g.* Carranza 2009), but being explicit in explanations of which individuals

are and which individuals are not included in measures of sexual selection will be important in allowing comparison of results between species (Klug *et al.* 2010b).

1.4 Variation in sexual selection within and between populations

As noted above, a key question in sexual selection is why it varies within and between lineages. Here I outline four broad areas (though not an exhaustive list) which may have an important role in explaining the observed variation in sexual selection in nature. These are i) the interaction between male competition and female choice, ii) variation in female choice due to the trade-off between good genes and compatible genes benefits; iii) the effect of environmental variation on sexual selection; and iv) plasticity and personality in sexual selection. Aspects of each are investigated in relation to sexual selection in red deer in the following chapters of this thesis: female choice (Chapter 2), environmental effects (Chapter 3), and plasticity and personality in sexual selection (Chapter 4).

1.4.1. The interaction between male competition and female choice

Although both male competition and female choice have been widely studied, the way in which they interact is still not clear (Qvarnstrom & Forsgren 1998; Wong & Candolin 2005). Where male competition and female choice co-evolve, they can have both positive and negative effects on each other (Berglund & Rosenqvist 2001; Candolin 1999; Doutrelant & McGregor 2000; Kangas & Lindström 2001; Wong & Candolin 2005). A recent review has indicated that whilst positive interactions between male competition and female choice are more common in the literature, the two processes are frequently opposing, particularly where the mechanisms occur at different timesteps (for example, when females choose from a subset of males who have already competed to hold territories, Moore 1990a, Hunt *et al.* 2008). Male competition and female choice complement each other when those males that succeed in male competition also on average provide more benefits to females than other males in the population (Cox & Le Boeuf 1977, Qvarnstrom & Forsgren 1998). These benefits may be direct, such as ability to protect a female from injury by subordinate males (Le Boeuf & Mesnick 1991, Qvarnstrom & Forsgren 1998), or indirect, if mating with dominant males produces offspring of higher fitness; if for example, dominant males have dominant sons (Moore 1990b). This will be reinforced if dominant males exclude inferior subordinates by competition, so that females end up choosing from amongst only the 'best males': then females would mate with high quality males at reduced searching costs (Wong & Candolin 2005). To this end, females may incite competition between males and mate

with the winner, a phenomenon that has been recorded in several mammalian species (elephant seals: Cox and Le Boeuf 1977; bison: Wolff 1998; elephants: Poole 1989).

However, male competition and female choice do not always have a positive influence on each other. For example, if female preferences are not positively correlated with male dominance, female choice can dampen correlations between male mating success and dominance (Moore and Moore 1999). Male dominance may not always indicate high genetic quality or viability. For example, where there is an energetic cost to dominance such as large body size, dominant individuals might be more prone to starvation in bad conditions (Qvarnstrom and Forsgren 1998). Therefore, in a variable environment it might sometimes be more profitable for a female to switch preferences from dominant to subordinate males (Ward 1998). Similarly, frequency dependent costs and benefits might make mating with a dominant or subordinate male more beneficial at different population frequencies of a given male phenotype (Sinervo & Lively 1996).

Where both male competition and female choice occur in a population, measuring the total extent of sexual selection necessarily requires measuring selection resulting from both mechanisms; yet empirical studies frequently consider only one or the other (Hunt *et al.* 2009). Identifying and separating the two mechanisms to measure selection independently is possible in lab studies, but the unpredictable interaction between the two makes it more challenging in the wild (Qvarnstrom & Forsgren 1998; Wong & Candolin 2005). Identification of female choice in polygynous species dominated by male competition, such as the red deer studied in this thesis, can be particularly difficult if males constrain females' ability to choose mates. For example, male competition may physically prevent females from mating with preferred males (Jennions & Petrie 1997; Trail 1985; Wong & Candolin 2005) or result in courtship interference (Howard *et al.* 1997, Sparreboom 1996, Kangas & Lindström 2001), or female preferences may be overridden by male harassment (Petersson *et al.* 1999). Further, environmental scenarios may also prevent females realising their mating preferences: for example, in many rutting species, dominant males become exhausted towards the end of the rutting period, and females are forced to mate with subordinates (Hirotani 1994).

1.4.2 Good genes versus compatible genes trade-offs

Under a 'good genes' framework, all females are expected to show preferences for mating with the same male: the male which can provide the greatest additive genetic benefit to their offspring, as indicated by some trait. In contrast, under a compatible genes

framework the optimal male for each female will depend upon the female's genotype; therefore different females should be selected to prefer different males. This presents a paradox: given that not all females are genetically identical, for most females the highest quality male is not the most genetically compatible (Mays & Hill 2004). How then can female preferences for both the most genetically superior male and the most genetically compatible male be resolved? Various possibilities have been suggested: for example, the option presenting the greatest benefit for females may be dependent on social or ecological conditions. Alternatively, rules for mate choice may be hierarchical, or nested, so that females use ornamentation to limit their choice of males to a small group, and then choose the most genetically compatible male from amongst that group (Candolin 2003, Mays & Hill 2004). Similarly, if female competition is strong, dominant females may monopolise highly ornamented males, leaving subordinates to choose from amongst the less ornamented males using genetic compatibility as a criteria. Empirical work on mice has shown that females have preferences for both 'good genes' males, who scent mark more frequently (indicating dominance), and 'genetically compatible' males, *i.e.* with dissimilar MHC haplotypes, and give more weight to dominance than MHC dissimilarity (Roberts & Gosling 2003). Conversely, female preference for unrelated males has been demonstrated to override dominance ranks in the field cricket (Simmons 1991).

1.4.3 The effect of environmental variation on sexual selection

Selection is not necessarily the same in constant environments as it is under variable environments (Cornwallis and Uller 2009). This is both an advantage and a constraint of studying wild populations: such studies have the potential to investigate how fluctuations in ecological parameters affect sexual selection, but studies which only examine selection at one point in time or space are likely to neglect a potentially important source of variation (Griffith and Sheldon 2001). It is therefore important not to extrapolate studies under the assumption that selection and distributions of phenotypes are constant over spatial and temporal scales (Bussière *et al.* 2008).

1.4.3.1 Genotype by Environment interactions for fitness in sexual selection

Genotype by environment interactions for fitness (GxEs) exist when the fitness of particular genotypes is dependent upon the biotic or abiotic environment in which they are expressed (Lynch and Walsh 1998). Explicit tests of GxEs in sexual selection have only begun to receive interest in the last decade (Ingleby *et al.* 2010). Where they have been tested, which is mainly in male sexual traits, substantial evidence for them has been found

(reviewed in Ingleby *et al.* 2010). For example, in Soay sheep, Robinson *et al.* (2009) showed that under favourable conditions, individuals with bigger horns have greater lifetime reproductive success, but that the opposite is true in poor environmental conditions. Furthermore, GxE have also been shown for female mate preferences (Rodigruez and Greenfield 2003). GxEs present interesting theoretical challenges for sexual selection theory in terms of signal reliability, maintenance of genetic variation and population divergence which are discussed in section 1.5.1 below.

1.4.3.2 Aspects of the 'environment' which cause variation in on sexual selection

Ingleby *et al.* (2010) argue that the majority of research to date on GxE in sexual selection has focussed on the role of abiotic factors: for example, rearing temperature is known to be important in determining adult signalling behaviour in the striped ground cricket, *Allonemobius socius* (Olvido and Mousseau 1995). However, the biotic environment is also likely to be important because it is subject to more rapid change (Wolf *et al.* 1999). For example, Kent *et al.* (2008) found significant GxEs in cuticular hydrocarbons with changes in social competitors. Despite this, few studies have explicitly tested for GxE in sexual traits with biotic factors (Ingleby *et al.* 2010).

However, it has long been known that the demographic or environmental setting in which selection acts is a key determinant of the strength of sexual selection. A substantial literature exists on how both abiotic and biotic factors - such as the number and availability of mates, the number and strength of competitors and climate - influence the strength of selection, such that under certain conditions the differences between successful and unsuccessful male genotypes are reduced or even reversed (for details, see Chapter 3). For example, several studies demonstrate that the success of subordinate or less successful males in male competition to gain breeding opportunities depends upon the density and age structure of competitors (Reichard *et al.* 2004a, Stevenson and Bancroft 1995, Bonenfant *et al.* 2004). Much of this literature has emerged from decades of research into sexual dimorphism and why different populations have different mating systems (Emlen and Oring 1977, Clutton-Brock and Harvey 1978). However, researchers have now also begun to consider why sexual selection varies within populations, between and even within breeding seasons (Kokko and Rankin 2006, McLain *et al.* 1993, Reichard *et al.* 2008, Kasumovic *et al.* 2008). There is still however a need to incorporate multiple environmental factors and their interactions in such studies (see Chapter 3). Although not explicit tests of genotype by environment interactions, such studies provide important insights into both the abiotic and biotic factors likely to influence the fitness of male

genotypes under different environments, and therefore detailed information of how spatial and temporal heterogeneity in sexual selection are likely to underlie genotype by environment interactions. For example, Gosden and Svensson *et al.* (2008) showed a highly variable mosaic of sexual selection on male body size in *Ischnura elegans* across a coastal to inland habitat gradient with changes in density of female colour morphs and local female body sizes.

Kin structure may also play an important role in the effects of environmental variation on sexual selection, because of changes in the costs and benefits of interactions between individuals. For example, in white winged choughs, *Corcorax melanorhamphos*, stable groups of cooperatively breeding relatives break down under extreme droughts, increasing the reproductive opportunities for unrelated individuals and so resulting in changes in the opportunity for sexual selection (Heinsohn *et al.* 2000).

1.4.3.3 Condition dependence

Williams (1966, 1978) suggested that the expression of sexual ornaments is dependent upon male condition. As resources are allocated to sexually selected traits, this depletes the resources available for other traits: for example, producing and displaying traits may have energetic costs (such as colouration of guppies, Endler 1983), or traits may impede foraging and therefore reduce condition (*e.g.* Moller 1989). Individuals in better condition are expected to have a greater pool of resources to draw from, so where traits are costly they are predicted to be condition-dependent (Rowe and Houle 1996). There is considerable empirical support for sexually selected traits being condition dependent (reviewed in Johnstone 2008).

One common, but sometimes overlooked, consequence of condition dependence in sexually selected traits is that traits will be highly sensitive to changes in environmental conditions (Cockburn *et al.* 2007). Consequently, the selective landscape may vary both temporally and spatially with environmental conditions, with the environment driving shifts in phenotypic optima. For example, female choice can vary spatially with predation risk (Stoner and Breden 1987). In male superb fairy wrens, *Malurus cyaneus*, Cockburn *et al.* (2008) showed that selection on a condition-dependent trait, early moulting, was stronger in years of high summer rainfall, in which more males were able to moult early. Further, sexually selected traits are sometimes influenced more by early environmental conditions than genetic effects: Griffith *et al.* (1999) showed that resemblance between

fathers and sons in sexual ornaments in house sparrows was due more to post-hatching environmental effects than genetic effects.

1.4.4. Plasticity and personality in sexual selection

1.4.4.1. Plasticity in sexual selection

Phenotypic plasticity, a change in phenotype in response to a change in environment, is widely observed in non-sexual traits (Pigliucci 2005). In sexual selection, plasticity is mainly considered in terms of condition dependence, but it is also worth considering that different individuals may respond differently to changes in condition, or may respond in unpredictable ways. For example, under poor conditions individuals in good condition might delay reproduction until conditions are more likely to result in success, whilst individuals with low chance of survival may attempt to reproduce (Cornwallis and Uller 2009). In general, in behavioural ecology, individual behaviour is assumed to be plastic so that an organism can adapt its behaviour to the optimal value in a given set of circumstances (Sih *et al.* 2004). Exploring patterns of within-individual trait expression may therefore reveal the costs and benefits of expressing those traits (Griffith and Sheldon 2001). However within-individual variation is also an important confounding factor in estimating, for example, heritability and selection on traits. Given that the effect of environmental variation on sexual traits may differ between individuals, characterizing an individual's expression of a trait at any one point may underestimate informative components of variation.

Displays and acoustic signals might be expected to be highly plastic signals, given their transient nature allows the potential for substantial variation, and interactions with partners or competitors will vary as a result of environmental or demographic processes (Endler and Rojas 2009). For example, male spring peepers (*Pseudacris crucifer*) modify the amplitude and type of calls depending upon the calls of their neighbours (Marshall *et al.* 2007.). Further, given the predation risk incurred through sexual signalling, males may be plastic in their displays according to current predation risk: Godin (1994) showed that male guppies switched from display mating tactics to sneaking under high predation conditions. Male guppies have also been shown to adjust mating tactics in response to a reduction in light levels (Chapman *et al.* 2009). This latter study illuminates our understanding of how guppies may respond to anthropogenic changes in the environment.

A number of studies have also investigated phenotypic plasticity in female mating preferences (*e.g.* Qvanstrom *et al.* 2001, Chaine and Lyon 2008, Milner *et al.* 2010,

Lehtonen *et al.* 2009). For example, the preference of female collared flycatchers for male forehead patch size varies with the time of breeding, presumably because the relative importance of the benefits indicated by male forehead patch size varies over the season. This trait is a particularly interesting example of plasticity, because male expression of the trait is also plastic: the forehead patch size decreased within individual males within years over the breeding season, and the amount by which it shrank was related to the initial size of the patch (Griffith and Sheldon 2001).

1.4.4.2. Personality in sexual selection

Personality, consistent individual differences in behaviour (Dingemanse 2009), is a burgeoning field in sexual selection. Behavioural consistency may be subject to sexual selection if being consistent in expression of some behavioural trait is costly and therefore indicates condition, either in male-male competition or mate choice. This will be particularly true if it is costly for males to maintain aggression or displays when environmental conditions deteriorate (Schuett *et al.* 2009, Byers 2007, Botero *et al.* 2009). For example, in male tropical mockingbirds, *Mimus gilvus*, syllable type consistency is linked to dominance status and reproductive success (Botero *et al.* 2009), and more consistent male songs elicit more aggressive responses from other males in great tits (*Parus major*, Rivera-Gutierrez *et al.* 2010). Other than consistency, evidence is also increasing that females might choose males for particular personality traits (Schuett *et al.* 2009, for example, boldness in Trinidadian guppies, Godin and Dugatkin 1996). Personality has also been applied to the concept of consistent differences between females in choosiness: individual females have been shown to be consistent in inclination to mate (Forstmeier 2004, 2007) and in time allocation to preferred male in mate choice trials (Forstmeier and Birkhead 2004).

1.4.4.3 The interplay between personality and plasticity: individual differences in plasticity

The interaction between personality and plasticity offers exciting new insights into the maintenance of variation within and between individuals, and to what extent this variation is adaptive (Dingemanse *et al.* 2010). The ‘individual reaction norm’ approach is increasingly being used to describe differences in phenotype with environmental change within and between individuals (Via *et al.* 1995, Pigliucci 2005). Extending this to behavioural traits, by relating individual levels of a behavioural trait to changes in context, we can assess not only population level average plasticity in the trait but also the

behavioural plasticity of individuals in response to changes in environment (Dingemanse *et al.* 2009). Further, decomposing trait variation in this way allows us to explore how and whether selection acts on these trait components, for example whether individuals which are more plastic in response to changes in environmental conditions have higher fitness (Nussey *et al.* 2005a, Nussey *et al.* 2007, Pigliucci 2005, Dingemanse *et al.* 2009). Studies of selection on plasticity in wild vertebrates currently exist mainly for life history traits (e.g. Nussey *et al.* 2005a), but interest in this topic for behavioural traits is growing. For example, Ural owl females vary in the extent to which they adjust their level of aggression in nest defence in response to changes in food conditions, and fierce nest defenders have more recruits (Kontiainen *et al.* 2009), although it is not known whether individuals which are more or less plastic differ in recruit success. Such analyses could provide important insights in the field of sexual selection. For example, as discussed above, behavioural consistency may be a target trait of female choice; conversely, in variable environments females may benefit by choosing males with high breeding values for plasticity.

1.5 The consequences of sexual selection

1.5.1. The lek paradox and maintenance of genetic variation for fitness

One major challenge to understanding the role of additive genetic benefits of female choice in sexual selection is the prediction that, as a consequence of directional mate choice, additive genetic variance for the male trait under selection should be eroded (the 'lek paradox', Taylor and Williams 1982, Rowe and Houle 1996). This will preclude females from gaining additive genetic benefits for their offspring by mate choice. In fact, the lek paradox is a special case of a general unresolved problem in classical population genetics theory: under directional selection, genes that confer fitness advantages should be driven to fixation, such that heritable genetic variance for fitness is depleted (Charlesworth 1987). Various explanations have been proposed to explain the maintenance of genetic variation for fitness generally. Further, in the case of directional mate choice, compatible genes and other non-additive genetic benefits of female choice have been proposed as a mechanism to explain the maintenance of genetic variance in sexually selected traits under female choice (Neff and Pitcher 2008, Kotiaho *et al.* 2008a, Hoffman *et al.* 2007, Reid *et al.* 2007). However, although this has produced some interesting avenues of research (*e.g.* Hoffman *et al.* 2007, Reid *et al.* 2007), it is worth remembering that under compatible genes models females differ in their preferences for particular males and so there is no directional selection on male traits, and hence no lek paradox (Kotiaho *et al.* 2008a).

Generally, explanations of the maintenance of genetic variation for fitness depend upon either fluctuating selection or mutation-selection balance (Tomkins *et al.* 2004). The genic capture hypothesis argues that both life-history and sexually selected traits are affected by a large number of alleles, because they are dependent upon condition and condition will be influenced by any allele which affects the ability of individuals to acquire and use resources (Rowe and Houle 1996, Tomkins *et al.* 2004). Therefore, any trait which is dependent upon condition will present a large target for mutations, and any mutation which affects condition will also affect sexually selected traits, increasing the variation in these traits.

GxE interactions have also been suggested as an explanation for the maintenance of genetic variation if no single genotype is superior to all others in all environments (ecological cross-over, Gillespie and Turelli 1989, Turelli and Barton 2004). This can maintain genetic variation if there is either spatial variation in selection and dispersal or temporal variation in selection and overlapping generations (Gillespie and Turelli 1989, Ellner and Hairston 1994, Danielson-Francois *et al* 2005, Kokko and Heubel 2008). The role of environmental variation in the maintenance of genetic variation has been investigated reasonably extensively in non-sexual traits. For example, Wilson *et al.* (2006) showed additive genetic variance in birth weight in sheep was under strong selection, but that additive genetic variance was greater in years when survival was high. When survival is high, selection is also at its weakest, so that there exists a negative covariance between genetic variance and selection, thereby constraining responses to selection. Charmantier and Garant (2005) conducted a meta-analysis of the effect of environmental stress on genetic variation in wild populations. Although the majority of traits reviewed showed no significant change in estimates of heritability under different environmental conditions, the authors found a significant decrease in heritability under poorer conditions, and that this effect was greater for morphometric traits than life-history traits.

In sexual traits that are targets of female choice, the potential for GxE interactions to explain the maintenance of genetic variation is hampered primarily by the problem that offspring may experience a different environment to the one in which their father's traits were selected. For example, Mills *et al.* (2007b) showed that in bank voles, male dominance (determined by testosterone) was heritable under stable conditions but was no longer heritable from father to son if the rearing environment deteriorated between generations. Kokko and Heubel (2008) have shown theoretically that GxEs can maintain genetic variation substantial enough to maintain indirect benefits of female mating

preferences, but only under the right amount of mixing by individuals between environments. The role of environmental variation and temporally and spatially fluctuating selection in the maintenance of genetic variation of sexual traits is a key area of future research (Ingleby *et al.* 2010).

As mentioned, as well as the role of condition dependence and GxE in the maintenance of genetic variation, some authors have proposed that female choice for non-additive genetic benefits could contribute to the resolution of the lek paradox (Neff and Pitcher 2008, Kotiaho *et al.* 2008a, Hoffman *et al.* 2007, Reid 2007). For example, Reid (2007) showed that female song sparrows prefer males with larger song repertoire sizes;these males have lower coefficients of inbreeding (*f*) (Reid *et al.* 2005). Further, *f* was correlated with a male's average relatedness to the female population, so that by choosing to mate with a male with a large repertoire size, females gained benefits of producing more outbred offspring. This implies that directional selection on a trait via female choice does not have to result in depletion of genetic variance for the trait. Strictly, whether the benefits to female song sparrows of mate preferences for large repertoire size are additive or non-additive is debatable (Kotiaho *et al.* 2008a, Puurtinen *et al.* 2009), but this should not affect the outcome for the lek paradox. However, a number of problems do exist with current arguments of how preferences for non-additive genetic benefits can resolve the lek paradox. For example, Hoffman *et al.* (2007) argue that the lek paradox is resolved in Antarctic fur seals because females prefer males with high heterozygosity which are unrelated, and therefore females are choosing a trait a) with low heritability and b) for which preferences will differ between females. However, it is difficult to understand what benefits exist to choosing males with high heterozygosity given the low heritability will limit the genetic benefits of this trait. Further, as I have already pointed out, if females prefer different males then there is no directional selection for female choice and there is no expected depletion of genetic variance, hence no lek paradox (Kotiaho *et al.* 2008a).

1.5.2 Population genetic structure, inbreeding and relatedness

The genetic structure of a population can both influence, and be influenced by sexual selection processes. For example, much theoretical and empirical work has been directed at the question of whether individuals avoid mating with relatives (inbreeding). Inbreeding increases the probability that an individual is homozygous for alleles identical by descent and this is expected to result in reduced fitness (inbreeding depression, Charlesworth and Charlesworth 1999, Keller and Waller 2002). Inbreeding depression in the wild is common and can be of substantial magnitude (reviewed in Keller and Waller 2002). For

example, in collared flycatchers, the number of offspring recruited to a nest where f=0.25 was reduced by 94% compared to an outbred nest (Kruuk *et al.* 2002a). Individuals are therefore predicted to avoid mating with close relatives, through dispersal or delayed maturation, but also by mate choice behaviour, such as recognising and avoiding kin as mates or extra-pair or carrying out extra-group copulations (Pusey and Wolf 1996, Pusey *et al.* 1987, Foerster *et al.* 2003, Lehmann and Perrin 2003, Berger *et al.* 1997, Reid *et al.* 2011).

Although the link between philopatry, dispersal and inbreeding has been given substantial theoretical attention (Chesser 1991, Sugg *et al.* 1996), increasingly complex cases of genetic structuring are appearing in the empirical literature (*e.g.* Double *et al.* 2005). For example, dispersal may be non-random with respect to genetic variance for traits (Garant *et al.* 2005) or there may be associations between sibs: Szulkin *et al.* (2009) found an unexpectedly high proportion of brother-sister pairings in a wild great tit population, and suggested this resulted from social bonds formed in the nest. Further, simulations have shown that population structures which result in the probability of inbreeding being non-independent between generations can result in parent-offspring correlations in f (Reid and Keller 2010). In general, even in species with natal dispersal, the risk of inbreeding will always be increased if there is a non-random distribution of mates with respect to relatedness. Where dispersal is incomplete, we therefore expect the evolution of other inbreeding avoidance mechanisms, such as kin recognition. However, even in species in which genetic structure could promote inbreeding, there is frequently a lack of evidence for inbreeding avoidance via kin recognition (*e.g.* Keller and Arcese 1998). Kin selection may be important in resolving this paradox, as non-random mating with respect to relatedness can have important consequences for the opportunity for kin selection (Cornwallis *et al.* 2009). Inbreeding can be favoured through kin selection, as it increases the mating success of a relative, so long as it does not reduce the mating opportunities of that relative and the cost in terms of offspring viability does not outweigh the inclusive fitness benefits (Parker 1979, Smith 1979, Waser *et al.* 1986, Kokko and Ots 2006). Further, non-random mating can increase the co-ancestry of philopatric groups and so increase the potential for cooperative behaviours to evolve (Chesser *et al.* 1991, Rossiter *et al.* 2005, West *et al.* 2002). For example, in horseshoe bats, the tendency for female relatives to mate with the same male results in an increase in co-ancestry within roosting females (Rossiter *et al.* 2005).

1.6 Spatial autocorrelation and shared environment in evolutionary and behavioural ecology

In populations with genetic structure, particularly where this structure includes inbreeding or other processes that increase co-ancestry, related individuals will tend to associate in similar locations. In heterogeneous environments, kin association can lead to a problem in disentangling genetic and environmental effects, because relatives will experience similar environments (Kruuk *et al.* 2000, Coltman *et al.* 2003, Garant *et al.* 2004, Kruuk and Hadfield 2007). This is likely to cause upward biases in estimates of the heritabilities of traits.

Spatial autocorrelation is increasingly realised to be an important source of bias in evolutionary and ecological studies (Legendre 1993, Valcu and Kempenaers 2010). Although researchers are becoming aware of the need to account for environmental effects in quantitative genetic analyses, in wild animal populations to date this has mostly been achieved through the inclusion of early life common environment effects such as nest box or maternal effects (with one exception, Van der Jeugd and McCleery 2002). In contrast, forestry and agricultural literature has shown how we can include more complex measures of spatial autocorrelation in estimates of quantitative genetic parameters, to account accurately for similarities between individuals due to location (Costa e Silva 2001, Cullis *et al.* 1991, Dutkowski *et al.* 2002). Further work is clearly needed to understand how we can correct for fine scale environmental sources of similarity between individuals in wild animal populations.

1.7 Red deer on Rum

In this thesis I examine the causes and consequences of sexual selection in a wild population of red deer. I examine the potential role of female choice in this population, how ecological factors affect variance in male mating success, plasticity in male signalling, how non-random mating affects relatedness and inbreeding and the consequences of relatives sharing environments on estimates of trait heritability. Full, relevant details of the study population in relation to the analyses are given in the methods section of each chapter, but here I present brief details of the history of study of this population, and explain some details of the life-history and mating behaviour of red deer.

1.7.1 Study population and area

The population of deer studied in this thesis live on the North Block of the Isle of Rum, a small (10,600ha) island 19km off the west coast of Scotland (see figure 1.1). Approximately 1000-1200 deer currently live on the island, based on helicopter counts in spring. Although red deer are native to the island, the native population became extinct in the eighteenth century, and the current population descends from a series of introductions between 1845 and 1972 (Clutton-Brock *et al.* 1982). The island was divided by the Nature Conservancy into five blocks for deer management purposes in the 1950s (see figure 1.2, Clutton-Brock *et al.* 1982). The North Block is an area of approximately 13km^2, consisting mainly of low-lying glens and one small hill (Mulloch Mor, see figure 6.1). The north of the study area is bounded by coastline from Kilmory Bay to another bay, Shamhnan Insir, and the majority of deer feeding occurs along this coastline and along the river which runs through Kilmory Glen (see figure 1.3 and Chapter 6 for further details). Prior to 1972, the whole island was maintained at a uniform deer density, with an approximately a 14% annual cull (Clutton-Brock *et al.* 1982, Clutton-Brock *et al.* 2002). However, in 1972, the North Block was released from culling and the population was allowed to expand. The population currently stands at around 200 adult females. The release from culling was associated with increased male mortality and male dispersal, as well as skew in male reproductive success (Clutton-Brock *et al.* 1997) which is discussed in more detail in Chapter 3.

Figure 1.1: the location of the Isle of Rum and **figure 1.2:** map of the Isle of Rum showing the boundaries between the management blocks. The study area is Block 4. Taken from Nussey *et al.* (2006).

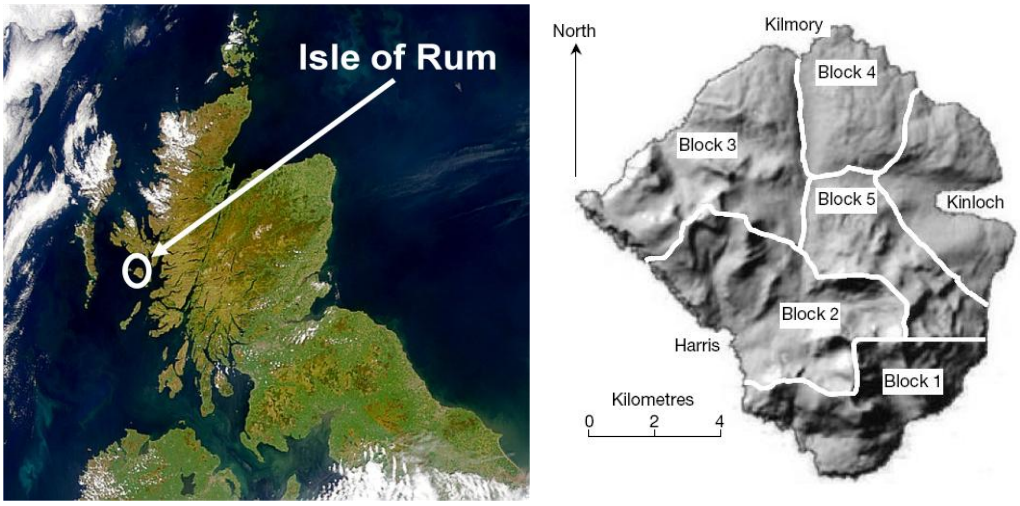


Figure 1.3: the north end of the study area, showing Kilmory Bay where the Kilmory river flows into the sea (Photo credit: Katie Stopher).

1.7.2 Red deer *(Cervus elaphus)*

The red deer is the largest living land mammal in Great Britain, distributed widely around Scotland and also other parts of the UK, particularly the Lake District, East Anglia and the South West (Ward 2005). They are polygynous, with marked sexual dimorphism (see figure 1.4) and a strong skew in male reproductive success (figure 1.5, Clutton-Brock *et al.* 1982). In the study population, there is philopatry amongst females whilst adult males disperse and live outside of the study area for the majority of the year (Albon *et al.* 1992, Clutton-Brock *et al.* 1997). On the mainland dispersal distances are not particularly far, but are again further for males than females: 3.3-7.4km for males and 1.9-3.5km for females (Daniels and McClean 2003), and the degree of population differentiation is similar between the study and mainland populations (Nussey *et al.* 2005b, Pérez-Espona 2008).

The mating season, or 'rut', runs from the middle of September to November. Adult rutting males return to the study area to 'rutting grounds', usually female-dominated areas of high quality grassland. They attempt to herd groups of females feeding on these grasslands into harems, and then compete to defend these harems and to mate with females within the harems when they come into oestrus. Male competition is strongly influenced by age, but other factors, including antler size and fighting ability are known to affect the outcome of competition (Clutton-Brock *et al.* 1979, Clutton-Brock *et al.* 1982, Kruuk *et al.* 2002b, Nussey *et al.* 2009). In general, only males of age five or above hold harems; young males (and sometimes senescent males) spend the majority of the time on the periphery of harems and may gain some reproductive success by sneaking matings when the more dominant stags are engaged in fighting (Clutton-Brock *et al.* 1982). Males use a wide variety of signals to display their dominance to other males: although roaring is by far the most obvious (see Chapter 4), males also thrash antlers and scent-mark landscape features such as posts. Challenges between males usually begin with a bout of roaring, and may escalate into parallel walks, and eventually full fighting (see figure 1.6). Fights impose a significant mortality risk; the sequence of assessment up to the point of fighting is therefore likely to allow males to assess dominance without taking this risk (Clutton-Brock *et al.* 1979). Oestrus periods in red deer usually last less than 24 hours and the majority of females mate only once (Clutton-Brock *et al.* 1982, Guinness *et al.* 1971). However, if a female fails to conceive while in oestrus, she may cycle and come back into oestrus after 18 days (Guinness *et al.* 1971). As the rut progresses, dominant stags, who do not feed during the rutting period, become exhausted and leave the rutting sites, and so females mating later in the rutting period are likely to mate with a younger, more subordinate male. Females cannot be forced to mate by a male, and whilst harem

membership is a good predictor of paternity, some females are thought to move between males when in oestrus (see Chapter 2).

Figure 1.4: a male, 'STUAR', with a female, 'BIT01' during the rut. (Photo credit: Martyn Baker)

Figure 1.5: distribution of lifetime breeding success gained in the study area by all deceased males which are known to the study population, and survived to 3 years of age. Shot males are excluded.

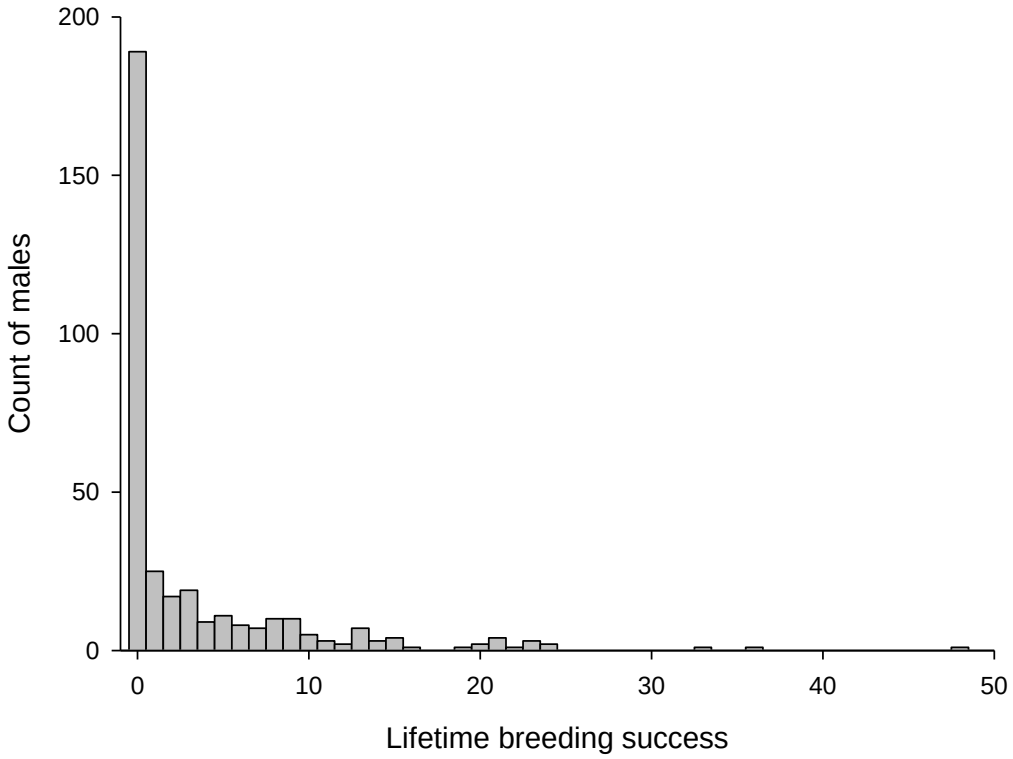


Figure 1.6:'TNG97' and 'TKN99' clash antlers in a fight. (Photo credit: Martyn Baker)

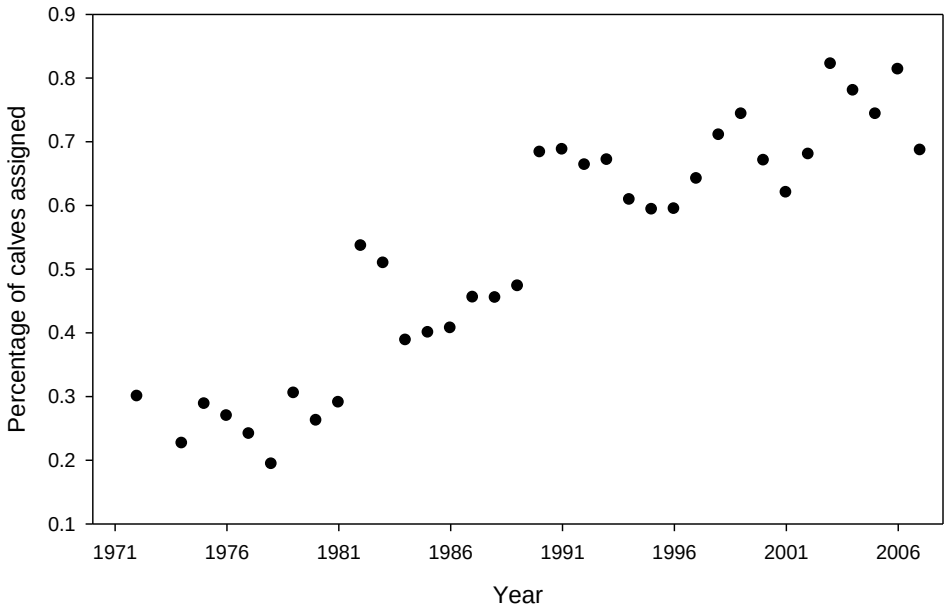
1.7.3 Data collection

The population has been studied since the 1960s, with intensive monitoring since 1971. Individual deer can be identified either by artificial markings - collars, tag and flashes- added just after birth, or natural idiosyncrasies such as ear shape and coat colouration. The main points of data collection relevant to this thesis are a) censuses, b) calving data and c) rut behaviour.

Censuses are conducted five times a month, by a field researcher walking a specified route and recording the identity and location of sighted individuals (Coulson *et al.* 1997). The census data used for analyses is usually taken from data collected in the months January-May, as at other times locations may be complicated by calving or mating behaviour (Moyes 2007). Although censuses are occasionally missed due to poor weather, the median number of censuses conducted over the study period (during January to May) is 25 per year. The recapture rate within these censuses is 1.0 (Fan *et al.* 2003). During the rut, more detailed censuses are undertaken: daily from the 15th September to 15th November. This provides detailed information on the location of individuals and harem membership, which is explained further in chapter 2. During these censuses, as well as from sustained periods of watching the rutting stands, observations are taken as to which females are in oestrus, using behavioural cues such as “chivvying” by males (see chapter 2 for more detail).

During the calving period, approximately May-July, detailed observations of pregnant females are undertaken in order to identify when and where calves are born. Calves are caught shortly after birth, and weighed, measured and marked. Over the entire study period, 64.2% of calves have been caught, with a success rate of over 75% since 2000. This provides accurate information on birth weight which can be corrected for age at capture (see Chapter 6). At this point, tissue samples (blood or ear punches) are taken for genetic analysis. Individuals born since 1991 have been genotyped at up to 15 highly variable microsatellites; prior to this, individuals were genotyped at up to 8 microsatellites. The rate of paternity estimation has increased steadily over time (see figure 1.7).

Figure 1.7. Changes in the percentage of calves assigned a father over time.



The use of genetic data to assign paternities has been a major advance in the study of this population. Whilst maternities are known through association with calves, paternities must be inferred by other means. Prior to genetic sampling, paternities were assigned using behavioural observations of which males a female associated with during the likely period of conception (Clutton-Brock *et al.* 1982). Studies have shown that whilst this is not an unreasonable proxy of paternity, it underestimates the true variance in male reproductive success (Pemberton *et al.* 1992). In addition to the use of genetic data, a recent study has provided more advances in our ability to accurately assign paternity of offspring. Walling *et al.* (2010) compared the use of the parentage program CERVUS (Marshall 1998) with two other programs, MasterBayes and COLONY2 (Hadfield *et al.* 2006, Wang and

Santure 2009), and argued that the increase in information which can be used by these programs, as well as the more appropriate method of estimating the confidence in paternity assignments, makes these programs more suitable for paternity estimation in the study population. MasterBayes combines use of both genetic and behavioural information, particularly harem membership by females and male age, both of which are known to be predictors of paternity (Clutton-Brock *et al*. 1982, Pemberton *et al*. 1992). COLONY2 combines paternity assignments with identification of clusters of half sibs, useful in red deer because of the high variance in male reproductive success. Use of these programs therefore allows parentage assignment to males which have not been sampled. Where a specific male cannot be assigned as the father of a half sib group, 'dummy sires' can be used to maintain estimates of variance in male reproductive success and to maintain paternal links within the pedigree for quantitative genetic analyses. Finally, Walling *et al.* (2010) demonstrates how the method employed by MasterBayes and COLONY of accepting parentage assignments at an individual, rather than population level, confidence precludes the acceptance of very poorly supported paternity assignments.

1.8 The objectives of this thesis

In Chapter 2, I make a detailed study of the movements of oestrous females during the rut, to investigate whether females move between harems when in oestrus to exercise mate preferences. I investigate the outcome of female movements among harems, asking whether the male whose harem is joined fathered the female's subsequent offspring, and whether females preferentially move to males which differ in age, harem size or relatedness. In Chapter 3, I examine the ecological factors which affect variance in male reproductive success, investigating the effects of demographic parameters such as sex ratio, synchrony of female oestrus timing and climate. I further assess whether standardized variance in male reproductive success, or 'opportunity for sexual selection' is a useful indicator of the strength of sexual selection by examining whether the ecological parameters which affect it also correlate with selection differentials of male rut start date, end date and antler mass. In Chapter 4, I examine intra-individual variation in acoustic behavioural reaction norms in male red deer. I examine how formant frequencies, an acoustic signal correlated with to body size, vary within and between males in response to the presence of an oestrous female, changes in harem size and duration of time spent rutting.

In Chapter 5, I examine whether female red deer mate with the same male in multiple years, and whether female relatives mate with the same male. I compare the observed

levels of these behaviours to those expected under a range of random mating scenarios, incorporating progressively more complex information on temporal, spatial and age constraints to mating. I also examine whether males rut in similar locations to their relatives, which would result in both females and males rutting in locations which were non-random with respect to relatedness. Finally, I compare the observed co-ancestry and inbreeding in the population to that expected under the aforementioned random mating scenarios, to investigate how re-mating between the same individuals, intra-lineage polygyny and genetic structure of rutting males are associated with the observed levels of relatedness and inbreeding in the population. In the final data chapter (6), I use measures of spatial autocorrelation, as well as a novel double-matrix method incorporating information on home range overlap, to account for environmental similarities between individuals in estimates of heritability from animal models of four example traits: rut home range size, spring home range size, birth weight and lifetime breeding success.

Chapter 2:

The red deer rut revisited: female excursions but no evidence females move to mate with preferred males

2.1 Summary

In polygynous mammals, most variance in male reproductive success is expected to result from competition between males, and the role of female behaviour remains poorly understood. Contests between red deer males during the annual rut are one of the most famous examples of male-male competition. However, anecdotal evidence suggests females in oestrus make substantial movements, changing harems and potentially disrupting the outcome of male contests. In other polygynous mammals, such movements have been interpreted as evidence of female mating preferences. Here we use 34 years of detailed observational data on wild red deer to show that 43% of oestrous females are found in different harems between successive observations, and that 64% of such females make substantial movements (up to 4km) when this occurs. Approximately 45% of these movements result in the male into whose harem a female moved fathering her offspring. We then test whether females move non-randomly with respect to male phenotype, consistent with the hypothesis that females move to mate with preferred males. Although in general, females were more likely to be found in larger harems and the harems of younger males after harem changes, these effects were not specific to oestrous females. Further, oestrous females were not more likely to be found in the harems of more or less related males. We therefore find little support for the idea that oestrous females move between harems to mate with a preferred male; as a result the reasons females make such extraordinary movements when in oestrus remain unclear.

2.2 Introduction

In non-lekking polygynous mammals, competition between males for access to receptive females was once assumed to explain a high proportion of the observed variance in male mating success (Clutton-Brock 1989). However, discrepancies between behavioural observations and molecular analysis of paternities have revolutionised our understanding of such mating systems, suggesting dominant males may gain more or less reproductive success than indicated by behavioural observations (Coltman *et al.* 1999, Twiss *et al.* 2006, Hughes 1998, Worthington Wilmer *et al.* 2000, Pemberton *et al.* 1992). This has important implications for the intensity of sexual selection (and therefore the potential for evolution), as well as the maintenance of genetic variation within a population (Coltman *et al.* 1999; Twiss *et al.* 2006, Hughes 1998, Hoffman *et al.* 2007). Where dominant males achieve less reproductive success than expected, this can be explained by the intensity of competition between dominant males, and/or the role of alternative male tactics (*e.g.* Reichard *et al.* 2004a, Coltman *et al.* 1999, Hogg *et al.* 1997); but female mating tactics may also play an important role *(*Hughes 1998, *e.g.* Travis *et al.* 1995).

Disentangling the roles of female behaviour and male competition in polygynous species in the wild has proved challenging, because the two can interact either positively or negatively, either reinforcing or weakening selection on male traits (Hunt 2009, Qvarnstrom & Forsgren 1998, Wong & Candolin 2005). Male competition can physically prevent females from exercising choice or limit choice to just a few dominant males (Jennions & Petrie 1997, Wong & Candolin 2005, Hunt 2009), but females can exert choice for dominant males by inciting competition between males and mating with the winner (elephant seals, Cox and Le Boeuf 1977; bison, Wolff 1998, elephants, Poole 1989). In several mammals, females show increased mobility during the breeding season (Byers *et al*. 1994, Relyea & Demarais 1994, Labisky & Fritzen 1998, San Jose & Lovari 1998, Dahle & Swenson 2003, Hoffman *et al.* 2007, Richard *et al.* 2008). These include the 'rut excursions' of roe deer, short term movements by females outside their home ranges, during which females visit one or more male territories to mate (San Jose and Lovari 1998, Lovari *et al.* 2008, Richard *et al.* 2008,); movements of female fur and grey seals among static males (Hoffman *et al.* 2007, Twiss *et al.* 2006); and the 'sampling' excursions of female pronghorn (Byers *et al.* 1994). Such excursion behaviours have the potential to disrupt the outcome of male competition and so influence the magnitude and direction of sexual selection, particularly if females have preferences for male traits other

than those favoured by male competition or if females use excursion behaviours to maximise the genetic compatibility of their mates (*e.g.* Hoffman *et al.* 2007).

Movements of breeding females during the breeding season are frequently interpreted as evidence of female choice (Byers *et al.* 1994, Twiss *et al.* 2006, Hoffman *et al.* 2007, Richard *et al.* 2008), but may be a result of a number of non-mutually exclusive processes (Clutton-Brock and McAuliffe 2009). As well as a female mating preferences for indirect benefits in terms of offspring fitness, including both good genes (*e.g.* pronghorn, *Antilocapra americana*, Byers *et al.* 1994) and genetic compatibility or inbreeding avoidance (*e.g.* fur seals, *Arctocephalus gazella*, Hoffman *et al.* 2007), movements could also represent active or passive attempts to escape from harassment by males (*e.g.* fallow deer, *Dama dama*, Clutton-Brock *et al.* 1992, Grevy's zebra, *Equus grevyi*, Sundaresen *et al.* 2007, red deer, *Cervus elaphus*, Carranza and Valencia 1999; but see Fisher and Lara 1999, in which the moderate speed of movements in wallaby females, *Onychogalea fraenata*, suggests they are not trying to escape harassment). Alternatively excursions may arise from mate searching behaviour, if males are sparsely distributed or cannot successfully court more than one female at a time (white-tailed deer, *Odicoileus virginianus*, Labisky & Fritzen 1998, roe deer, *Capreolus capreolus*, Lovari *et al*. 2008, *Capreolus capreolus*, Richard *et al.* 2008). Furthermore, if movements are not specific to oestrous females, such female movements could arise from a response to variation in environmental conditions such as weather (grey seals, *Halichoerus grypus*, Twiss *et al*. 2007). Determining which of these mechanisms underlies this female behaviour is important to understanding whether these movements are relevant to the evolution of male traits.

Despite the potential importance of female excursions during the breeding season, few studies of wild mammals have been able to explicitly link such behaviour with female receptivity; to examine whether females mate with the male they move to; or to compare the phenotypes of the males between which females move (Richard *et al.* 2008, but see Byers *et al.* 1994 and Hoffman *et al.* 2007 in which evidence is presented as to the phenotypes of males joined). All of these, but in particular the comparison of male phenotypes, are essential in order to test whether females move in order to mate with a preferred male. As such, in published reports, attempts to disentangle the reasons females move have frequently proved challenging or controversial (*e.g.* Hoffman *et al.* 2007 and Kotiaho *et al.* 2008b), and it has proved unclear whether movements are important

relative to male competition; this is critical for our understanding of how male competition and female mating tactics interact.

The present study

In this study, we use 34 years of detailed observational data on female and male location, female oestrus dates and male traits, as well as a detailed pedigree, from a wild population on the Isle of Rum, Scotland. We test whether the probability of females changing harem, and the distances they move in doing so vary with female oestrus state, whether movements around oestrus result in changes in the sire of a female's offspring, and whether females move in order to mate with a preferred male.

During the red deer mating season (or "rut"), males compete to obtain and defend harems of females, and to mate with these females as they come into a brief oestrus (24 hours, Guinness *et al.* 1971). During the rut, female home ranges are constricted, and most harem membership over the period is probably influenced by the availability of food and the location of relatives (Clutton-Brock *et al*. 1982). Previous work has shown that the outcome of competition between males for females is determined by male traits such as age, body weight, antler size, number of antler points and fighting skill, and that the outcome is important in explaining the distribution of reproductive success (Clutton-Brock *et al.* 1979, Kruuk *et al.* 2002b). However, anecdotal evidence suggests that when females are in oestrus they sometimes leave their current harem and move substantial distances to join the harem of a different male (Clutton-Brock *et al.* 1982). Our analysis builds on previous research on the long term study of a red deer population on the Isle of Rum, showing that, even during the most stable periods of the autumn rut, over 30% of females are in different harems between one day and the next and that females are able to move between harems despite attempts by males to prevent them (Clutton-Brock *et al.* 1982). In particular, to some extent, harems are re-assembled each morning, as at night, females leave the rutting grounds, which are predominately in low altitude areas, and move onto the lower slopes of the surrounding hills (Clutton-Brock *et al.* 1982), moving back down the slope at dawn. However, currently, the reasons for these changes in harem membership are unclear. There is little current evidence for female choice in wild populations of red deer (although see Carranza *et al.* 1995 for evidence of female choice for territory rather than male). However, a number of studies in semi-captive and farmed populations have argued that females express preferences for male traits such as antler size, or properties of the male roar (antlers: Bartos *et al.* 2007, Malo *et al.* 2005 ; roars:

Charlton *et al.* 2007a, Charlton *et al.* 2007b, McComb 1991, Reby *et al.* 2001, Reby and McComb 2003, Reby *et al.* 2010 but see Charlton *et al.* 2008).

In our study population, substantial data exists on male age, harem size and relatedness to females. Although no studies have yet demonstrated preferences for these characteristics in wild red deer they are all plausible candidates for male traits as targets of female choice. Females may prefer older males because their ability to survive demonstrates high genetic quality for viability (the age-based indicator mechanism: Hansen and Price 1995, Brooks and Kemp 2001) or because mating with inexperienced young males has a high risk of injury (Clutton-Brock *et al.* 1982). Females may prefer males holding larger harems as this can indicate male fitness (Gibson and Guinness 1990); or because they experience reduced per-capita harassment in larger harems (Clutton-Brock and McComb 1993, McComb & Clutton-Brock 1994). Finally, females may move from harems controlled by related males to mate with an unrelated, or less related, male to avoid costs of inbreeding. In recent literature there has been increasing understanding of the importance of genetically compatible mates on offspring fitness through the benefits of masking deleterious alleles (dominance) or via heterozygote advantage (overdominance) (reviewed in Coulson *et al.* 1998, Amos *et al.* 2001, Hansson and Westerberg 2002, Mays and Hill 2004).

In this study we aim to test whether oestrous females move between harems in order to mate with preferred males. We make the following predictions:

P1: Females will be more likely to change harem when in oestrus than at other times, and will move further, to locations outside their rut home ranges, in doing so.

P2: When females change harem during oestrus, the novel male will sire the females' offspring;

P3: Females changing harem in oestrus will do so non-randomly with respect to male phenotype, moving preferentially to the harems of older males, males holding larger harems and/or the harems of less related males.

2.3 Methods

Study population and data collection

Data was collected from a free-living red deer population living in the North Block of the Isle of Rum, off the west coast of Scotland, in which individuals of both sexes can be

recognized, either from artificial marks placed at birth or individual idiosyncrasies, such as facial shape, body shape, coat colouration or natural ear notches (Clutton-Brock *et al.* 1982). Individual life histories have been closely monitored in this population since 1972 (see Clutton-Brock *et al* 1982). Each year, during the rutting period (defined as 15th September to 15th November), daily censuses of the study area are conducted. The identities and locations (correct to the nearest 100m) of all females seen and all males that are with at least one female are noted during these censuses, and the identities of the females comprising the harem of each male are also recorded. It should be noted that these records provide a conservative estimate of female movement: because females are systematically recorded once per day, they may also move between harems within a day. The average harem size is 6.20 females, although this ranges from 1 to 78. Males holding harems range in age from 1-17, with a median of 7. Opportunistic sightings of behaviours indicating females are in oestrus are made throughout the rutting period (see below and Clutton-Brock *et al.* 1997). Oestrus periods in red deer usually last less than 24 hours and the majority of females mate only once (Clutton-Brock *et al.* 1982, Guinness *et al.* 1971). Behaviours indicative of oestrus are chivvying of females (harassment) by their harem holding male, a female being in an unusual location, or a female being mounted or served (*i.e.* the male was seen to ejaculate) or straining, as occurs after service (F. Guinness, pers. comm., Clutton-Brock *et al.* 1982).

During the rut, female home range sizes are constricted from those occupied at other times of the year (Clutton-Brock *et al.* 1982). The median core home range size (see below) during the rut is 37ha, although there is substantial variation (2-1557ha, see Chapter 6). Although this median home range size potentially encompasses multiple harems, stags invest considerable effort in herding groups of females, so that harems are typically distinct from one another (Clutton-Brock *et al.* 1982). However, some small movement of harems between and within days is likely to occur as grazing females move between resources; therefore a female's home range throughout the rut is generally larger than the area a harem occupies at any point.

During the calving season (approximately 20th May to 30th June) daily observations are made to identify the calving date of each female and monitor neonatal survival (Clutton-Brock *et al.* 1982). Calves are caught shortly after birth and tissue samples taken for genotyping. In addition, for possible sires not caught at birth, most have been sampled from cast antlers, or by chemical immobilization or post-mortem. Individuals born since 1991 were genotyped at up to 15 highly variable microsatellites; prior to this, individuals

were genotyped at up to 8 microsatellites. Paternities were assigned using the programs MasterBayes (Hadfield *et al.* 2006) and COLONY2 (Wang and Santure 2009) with greater than 80% individual confidence (see Walling *et al.* 2010 for full details).

The following were used as explanatory variables in our analyses:

Confidence of oestrus observations: One of three levels of confidence was assigned to each oestrus observation, depending on the behaviour on which the assignment was based. Confidence levels were 'possible' (the female being chivvied by a male more than other females in the same harem); 'likely' (the female being frequently, selectively and intensively chivvied, or being in an unusual location, see below); and 'definite' (the female being mounted, served, or straining). Where more than one oestrus date was recorded for a female, we used the oestrus date with the highest confidence attached. Where two possible oestrus dates were recorded with equal confidence, both observations were removed from the dataset (24 out of 2236 observations).

Day relative to oestrus: The number of days between the day of observation and the day on which the female was in oestrus, grouped as: a) day of oestrus, b) day before oestrus, c) day after oestrus, d) other day in the rut.

Peak rut: This was included to account for changes in the stability of harems and change in age structure as the rut progresses (Clutton-Brock *et al.* 1982). It was treated as a two level factor denoting whether the day of observation was during the peak of the rut. Peak rut is defined as the period within an eleven day window around the average date of conception in any one year, where conception dates are calculated from the birth date of calves born following that rut, with gestation length taken to be 235±5 days (Clutton-Brock *et al.*1982).

Days between observations: The number of days between subsequent observations of a female. This was included as a covariate to correct for events in which a female was not seen on a daily census, which will increase the period and so theoretically, distance, which the female could move between observations.

Maximum temperature: this is recorded on Rum by Scottish Natural Heritage, and missing values were predicted using a regression equation from those on Tiree, a nearby island with more complete climate records, with which Rum temperatures are highly correlated (R=0.971, for more details, see Moyes *et al.* 2010). For remaining gaps, temperatures

were estimated from the mean temperature over the previous three days and subsequent three days (Moyes *et al.* 2010).

Rainfall: this was again recorded on Rum by Scottish Natural Heritage, and supplemented by a regression equation used to predict the rainfall from records taken at Rhubana, Inverness-shire (Moyes *et al.* 2010)*)*, with which Rum rainfall is again highly correlated (R=0.909). Data from Rhubana is less complete than Tiree; however, rainfall is much more locally variable than temperature and therefore it is more important to use the closest possible station proving adequate rainfall data.

Construction of rut home ranges

An average of 43.04±18.94 observations were available per female per year. Females with less than 6 recorded positions were excluded, removing 4.33% of the data. This is a little less than that recommended by Borger *et al*. (2006), who found 10 fixes collected over a few days per month were sufficient in roe deer and kestrels for accurate home range estimation using similar methods. However, visual assessment suggested this was a sufficient number of fixes to produce sensible home range shapes. Home ranges were calculated using kernel density estimation methods (Borger *et al*. 2006; Worton 1987; Worton 1989). The kernel method calculates boundaries based on the complete utilization distribution and can account for multiple centres of activity (Kenward *et al*. 2001; Worton 1989). The smoothing parameter, h, was selected using the reference bandwidth, h_{ref} method, as the least squares cross validation method performed poorly with home ranges with small numbers of fixes (as predicted by other studies, Seaman & Powell 1996). The h_{ref} method tends to over-smooth home ranges (Huck 2008), which also makes it a more conservative smoothing algorithm in this analysis (see below) than the least squares cross validation method. Home ranges were calculated in 'R' version 2.6.1 (R Development Core Team 2007) using the 'adehabitat' 1.6 package (Calenge 2006) and then imported to ArcMap. In this study, we used 65%, 85% and 90% isopleths. Borger *et al*. (2006) recommend using isopleths between 50 and 90%, as larger and smaller isopleths are subject to greater bias. Determination of whether female locations fell within calculated home ranges was done using the 'intersect point tool' in Hawth's Analysis Tools for ArcGIS (Beyer 2004), so that each position a female was recorded in was assigned a 0 if it fell outside the female's rut home range, and a 1 if it fell inside.

Statistical analysis

For this study, we use data from the ruts in years 1974-2007, excluding 2000 in which there is no oestrus data available. All statistical analyses were conducted using the statistical package 'R' version 2.8.1 (R Development Core Team 2008) or Genstat version 11.1 (Payne *et al.*2009). Model selection was based on sequential removal of variables with non-significant Wald statistics (Crawley 2007).

1. Female movements and their reproductive consequences

We used (generalised) linear mixed-effects models, (G)LMMs, to test how day relative to oestrus influenced a female's probability of changing harem between observations, and how far she moved in doing so. Harem change was modelled as a binary variable denoting whether a female was in the harem of the same male (1) or a novel male (0) compared to the last observation of that female. Observations where the identity of the previous or current male was unknown were not assigned values. The distance moved by a female between successive observations was measured as the distance in metres between the female's previous position (position to the nearest 100m) and the female's current position. Occasionally, the fact a female has travelled a long distance from her normal location is taken as an indication that she is in oestrus; this presents obvious circularities in our analyses, and such observations were therefore excluded from all analyses. Secondly, individual males do not rut for the entire season, but at some point terminate their rutting activities, typically as a result of defeat in a fight and/or exhaustion (Clutton-Brock *et al.* 1982). When this occurs, they are often observed to leave the rutting areas for a sustained period of time, or even for the remainder of that season. Such male turnover events will result in females being found in a different harem because of exclusively male, not female, behaviour. We therefore excluded from analyses all observations in which the harem holder a female had been seen with initially was not holding a harem at the subsequent observation of that female.

To test whether females were more frequently observed to change harems on the day of oestrus than at other times during the rut, as was suggested by earlier studies conducted on this population (Clutton-Brock *et al.* 1982), we fitted a GLMM of harem change with a binomial error structure. Day since 1st September and female identity were included as random effects. Fixed effects were day relative to oestrus, year of observation, confidence level of oestrus observation, peak rut, daily rainfall and minimum temperature.

To test whether females in oestrus made movements of unusual distance which resulted in them being associated with a different male, we fitted a LMM of the distance between the female's current and previous location (to the nearest 100m). Distance moved was log-transformed and residual errors checked for normality. Again, day since 1st September and female identity were included as random effects. Fixed effects included were day relative to oestrus, change in male, year, confidence level, daily rainfall, minimum temperature, days between observations and peak rut. An interaction was fitted between day relative to oestrus and change in male to examine how the distances moved by females changing harem and not changing harem varied with oestrus state.

We also investigated how often the movements of females between harems in different oestrus states resulted in females moving away from their rut home ranges. This analysis was conducted using data from 2003 to 2007 only. The aim of this analysis was to determine whether the probability of a female being outside her 'rut home range' increased when the female was in oestrus. We first calculated the 'rut home range' for each female, using each position in which a female was recorded during the daily rut censuses. Each home range was calculated at three isopleths: 65%, 85%, and 90%. A 65% isopleth home range represents a core home range for each female (Moyes 2007), whilst 85% and 90% represent progressively more inclusive ranges, so that in a 90% home range, only 10% of a female's locations will fall outside of its boundary. We then determined whether female locations fell within calculated home ranges using the 'intersect point tool' in Hawth's Analysis Tools for ArcGIS (Beyer 2004). These data were collated for all females as proportion data grouped according to whether a female changed harem since her previous observation, and her day relative to oestrus. Because of the nature of the home range sizes, proportions were highly skewed and therefore the response variable was transformed using an arcsine transformation (Crawley 2007). The data were analysed using a linear model (LM), with transformed proportion of females outside their rut home range as a response variable, and day relative to oestrus, change in male, year and confidence as explanatory variables.

To determine the reproductive outcome of movements by oestrous females, we tested whether paternities were gained by males as a result of such movements. To test this, we defined a subset of females as 'movers' if they changed male and moved further than the median distance moved by females that changed males when in oestrus. Females for which we did not have an observation on both the day before and the day of oestrus, or for which the male they moved to was not known, were excluded. We then calculated the

proportion of offspring born to females which a) changed harem in oestrus and b) changed harem and were defined as 'movers', that were sired by the novel male.

2. Analysis of male phenotypic traits and relatedness to focal female

To determine whether the likelihood of females changing harem was correlated with male phenotypic traits, we compared the trait values of males holding harems which females moved between. We considered age, harem size and relatedness to the focal female as phenotypic traits of the male. Male ages were known for males which were born into the population and therefore were observed as calves, or, for males not born to the population estimated from appearance, including traits such as body and antler size. Recent work has demonstrated that the pattern of male reproductive success with age is similar for males with known ages and those with ages estimated from appearance, indicating our estimates of the age of such males are likely to be accurate (Chapter 3). Harem sizes were known from rut census data. Relatedness was calculated using the reconstructed population pedigree using the kinship package in R (Atkinson 2008). Each female movement was considered a contest between a pair of males, with the male into whose harem she moved from scored as '0' and the male whose harem she moved into scoring '1'. One of each pair was randomly assigned as a focal male, and then we used a GLMM, with a binomial error structure, to consider the probability of the focal male winning the contest given the difference in trait values between the pair (*i.e.* trait value for focal male minus trait value for non-focal male). Identities of the two males in each pair were included as random effects in all models. Fixed effects included in the model were day relative to oestrus (as before), differences in the above male traits, as well as year of observation and confidence of observation. We fitted an interaction between day relative to oestrus and the difference between male phenotypes to test whether female preference varied with oestrus state. The random selection of a focal / non-focal males and GLMM analyses were repeated 1000 times to ensure the random selection of focal/non focal male was not driving any significant results. Due to the computational time required to do this, it was not possible to simplify these models using stepwise deletion of non-significant terms. Since harem size is known to be closely correlated with age (Nussey *et al.* 2009), we also ran similar models in which the difference in each male trait was tested separately, without the other traits. These models included the same other fixed effects as before, and again were tested with and without an interaction term fitted between difference in male trait and female oestrus state. Again, the random selection of a focal male/ non-focal males and GLMM analyses were repeated 1000 times.

In a second analysis of male phenotype, we asked whether females changing harem moved further depending on the difference between male phenotypes, and whether this changed when females were in oestrus. This analysis was an extension of the previous analysis of distance moved by females, with explanatory variables as above, and also, fitted as a fixed effects, the difference between male trait values for age, harem size, and relatedness to the focal female, as the trait value of male joined by the female minus the trait value of male left by the female, and the interaction between the difference in male trait and female oestrus state. Female identity and the identity of the pair of males which females moved between were fitted as random effects.

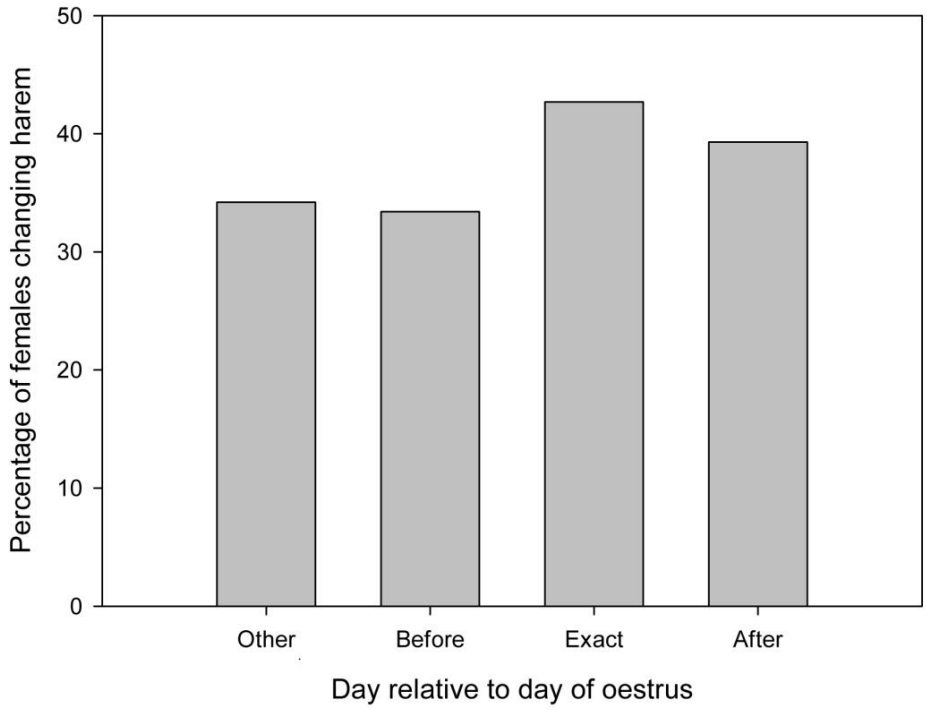
2.3 Results

Harem changes, female movements and reproductive consequences

Females were more likely to change harem between observations when in oestrus than either the day before oestrus, the day after oestrus or other days in the rut (figure 2.1, in a GLMM of harem change, with a binomial error structure, with days since 1st September and female identity as random effects, $F_{3,15707.0}$=5.00, p=0.002). Combining 'day after', 'day before' and 'other days', we found that females were more likely to change harem when in oestrus than on all other days in the rut (42.7% of females changed harem on the day of oestrus compared to 34.3% on other days in the rut: $F_{1,15745.1}$=13.36, p<0.001). However, harem change on the day after oestrus and the day before were both not significantly different than other non-oestrus days in the rut ('day after' versus 'other', 39.3% versus 34.3%, $F_{1,15194.9}$=1.54, p=0.215, 'day before' versus 'other', 33.4% versus 34.2%, $F_{1,19185.8=}$0.06, p=0.801).

The likelihood of females changing harem also varied with year ($F_{29, 6511.7}$=4.59, p<0.001), and decreased as rainfall increased (Estimate=-0.009±0.002, $F_{1,15788.5}$=35.46, p<0.001). There was no effect of confidence of oestrus observation, timing in relation to the peak of the rut, or temperature (in the full GLMM of harem change- containing fixed effects of day relative to oestrus, year of observation, confidence level of oestrus observation, peak rut, daily rainfall and minimum temperature, as well as random effects of days since September 1st and female identity- confidence $F_{3,5588.5}$=1.83, p=0.139, peak rut $F_{1,14795.2}$=1.01, p=0.316, minimum temperature $F_{1,13932.4}$=2.23, p=0.136).

Figure 2.1: percentage of females changing harem by day of observation relative to oestrus.

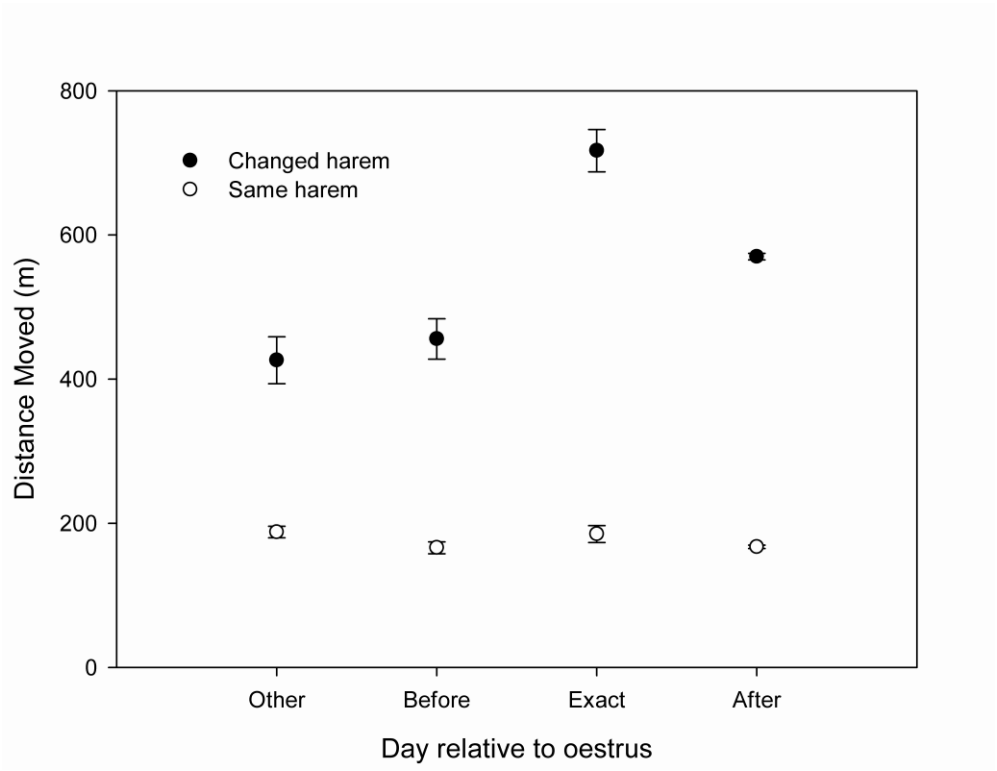


Females that changed harems tended to move further between observations when they were in oestrus than at other times in the rut (figure 2.2). In a LMM of distance moved by females between successive observations, the interaction between day relative to oestrus and whether a female changed male on the distance moved by a female was highly significant (day relative to oestrus by change in male interaction: $F_{3,4993}=6.55$, $p<0.001$, table 2.1). As figure 2.2 shows, females that changed harem moved further when in oestrus than at other times in the rut, but females remaining within the same harem moved similar distances whether in oestrus or not. The distances females moved also varied between years, and were positively correlated with the number of days between observations of that female (see table 2.1). Females moved shorter distances between observations during the peak of the rut, but moved further when rainfall was higher and when the minimum daily temperature was warmer (see table 2.1)

Table 2.1: results from the minimum adequate behavioural oestrus dataset movement model, in which the response variable is distance moved: a LMM, based on 14748 observations. The estimated effect size and standard error from the LMM are given, with F values and p values tested based on type II sums of squares. Effect sizes for each year are not shown.

Term	Estimate	S.E.	F value	d.f.	P
Intercept	5.504	0.102	2894.91	1, 9323	<0.01
Day relative to oestrus			7.16	3,4993	<0.01
Day relative to oestrus (before)	0.143	0.164			
Day relative to oestrus (exact)	0.543	0.129			
Day relative to oestrus (after)	0.283	0.141			
Male			1290.51	1, 4993	<0.01
Male (same)	-1.206	0.034			
Year			4.09	1, 4993	<0.01
Days between observations	0.097	0.020	24.66	1, 4993	<0.01
Peak rut			13.09	1, 4993	0.02
Peak rut (TRUE)	-0.124	0.034			
Rain	0.006	0.001	17.28	1, 4993	<0.01
Minimum temperature	0.011	0.005	4.26	1, 4993	0.04
Day relative to oestrus x Male			6.55	3, 4993	<0.01
Day relative to oestrus (before) x Male (same)	-0.347	0.203			
Day relative to oestrus (exact) x Male (same)	-0.591	0.171			
Day relative to oestrus (after) x Male (same)	-0.435	0.178			

Figure 2.2: plot showing the effects of day relative to oestrus and whether the female had changed harem on the distance a female moved between successive observations. Day relative to oestrus is grouped into day of oestrus, day before oestrus, day after oestrus and other days in the rut.



Females were also more likely to be outside their rut home range when in oestrus than at other times in the rut: there was a interaction between day relative to oestrus and whether a female changed harem on the likelihood of being outside her 'rut home range' ($F_{3,102}$=6.4183, p=0.0005, see figure 2.3a and table 2.2). However, this was only true for rut home ranges calculated at a 65% isopleth. Again, grouping day relative to oestrus into two factor levels showed that females that changed male were more likely to be outside their home range when in oestrus than at other times in the rut (t=4.899, d.f.=54, p<0.0001), but were not more likely to be outside their home range on the day after oestrus than at other times (t=-1.488, d.f.=54, p=0.142, see figure 2.3a). This indicates that females return to their rut home range after oestrus. Although not significant, the patterns were broadly similar for home ranges calculated at 85% and 90% isopleths (see table 2.2, figure 2.3 b and c).

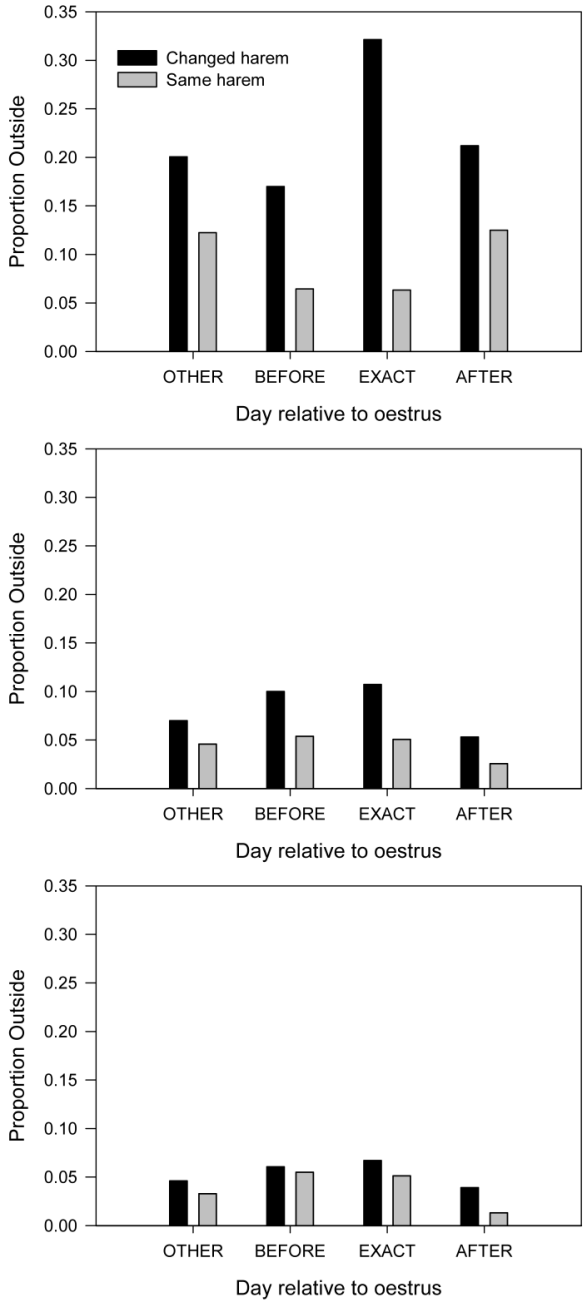
Table 2.2. Results from a LM in which the response variable was the proportion of females outside their home range. Results reported here are F values and p values tested based on type II sums of squares for a LM, testing the effects of day relative to oestrus and whether females changed harem on the proportion of females outside their rut home range. Results are given for home ranges calculated at three isopleths: 65%, 85% and 90%.

Isopleth %	Term	d.f.	F value	p value
65	Day relative to oestrus	3,109	6.9301	0.0003
	Male	1,108	23.454	<0.0001
	Day relative to oestrus x Male	3,102	6.4183	0.0005
85	Day relative to oestrus	3,110	1.4927	0.2210
	Male	1,109	6.4469	0.0126
	Day relative to oestrus x Male	3,103	1.1514	0.3321
90	Day relative to oestrus	1,110	1.6365	0.1855
	Male	1,109	1.7325	0.1910
	Day relative to oestrus x Male	3,103	1.1934	0.3161

In analysing the reproductive consequences of female movements, we identified 40.5% (202 out of 499) of oestrous females considered in this analysis which were positively identified as being with a different male when in oestrus compared to the previous observation, 68.3% of which (106 out of 202) moved further than the median distance moved by females when changing harem. Of all females which changed harem when in oestrus, 95 produced a calf which could be assigned a sire from the pedigree, and of females which both changed harem and moved further than the median distance, when in oestrus, 53 produced a calf which could be assigned a sire from the pedigree. For individuals that changed male when in oestrus, the assigned sire matched the male the

female moved to when in oestrus in 43.2% (41 of 95) cases. For individuals which changed male and moved further than the median distance the assigned sire matched the male the mother moved to when in oestrus in 45.0% (24 of 53) cases.

Figure 2.3: barchart showing the proportion of females outside their rut home range grouped by day relative to oestrus, plotted according to whether the female changed harem. Black bars refer to females changing harem, grey bars refer to females that remained with the same male. Results shown are for a) a 65% isopleth rut home range, b) an 85% isopleth rut home range and c) a 90% isopleth rut home range.



Male phenotypic traits and relatedness analysis

Throughout the rut, females changing harem tended to do so from older to younger males (independent of harem size, average estimated effect of age difference= -0.325, average standard error=0.034, average Wald value= 94.054, average p value<0.001, see figure 2.4a, table 2.3a) and smaller to larger harems (independent of male age, average estimate= 0.104, average standard error=0.009, average Wald value=122.103, p<0.001, see figure 2.5, table 2.3a.), but there was no association with relatedness of the male to the focal female. In the full model, testing for interactions between male traits and female oestrus, there was no significant interaction between day relative to oestrus and female preferences: the effects of male phenotype on female harem changing were the same for oestrous and non-oestrous females (see table 2.3b). However, when male age was considered without the other traits in the model, there was a weak trend towards an interaction between female oestrus state and difference in male age (average Wald value= 3.110, average p value=0.102); which would indicate that females in oestrus were less likely to move to younger males (see figure 2.4b). This discrepancy between models including all male phenotype differences and the model including only male age difference is likely to be due to correlations between harem size and male age, and so the result should be treated with caution- this finding does not provide evidence of mating preference. Considering harem size or relatedness to the female without other male traits in the model had no difference on our findings (in such models, main effect of harem size, average Wald value=112.794, average p<0.001, main effect of relatedness, average Wald value= 0.162, average p=0.749).

Figure 2.4: the probability that the focal male 'gained' the female against the difference in age between the focal and non-focal male: each colour of points and coloured regression line relates to the data generated from one of 1000 samples in which the focal male was randomly selected from the pair of males to be compared (see text).

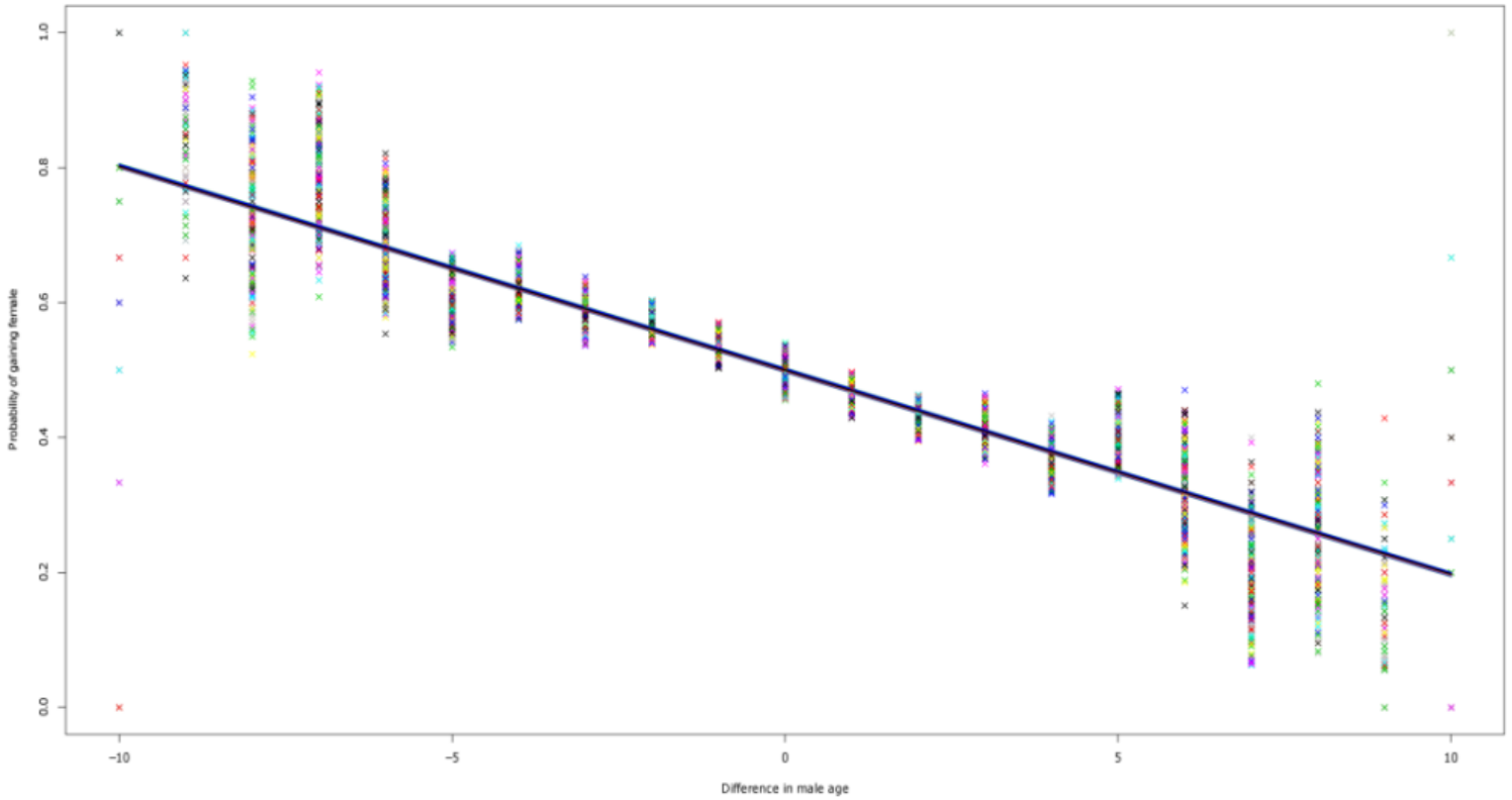


Figure 2.4b: just for females in oestrus, the probability that the focal male 'gained' the female against the difference in age between the focal and non-focal male. Each colour of points and coloured regression line relates to the data generated from one of 1000 samples in which the focal male was randomly selected from the pair of males to be compared (see text).

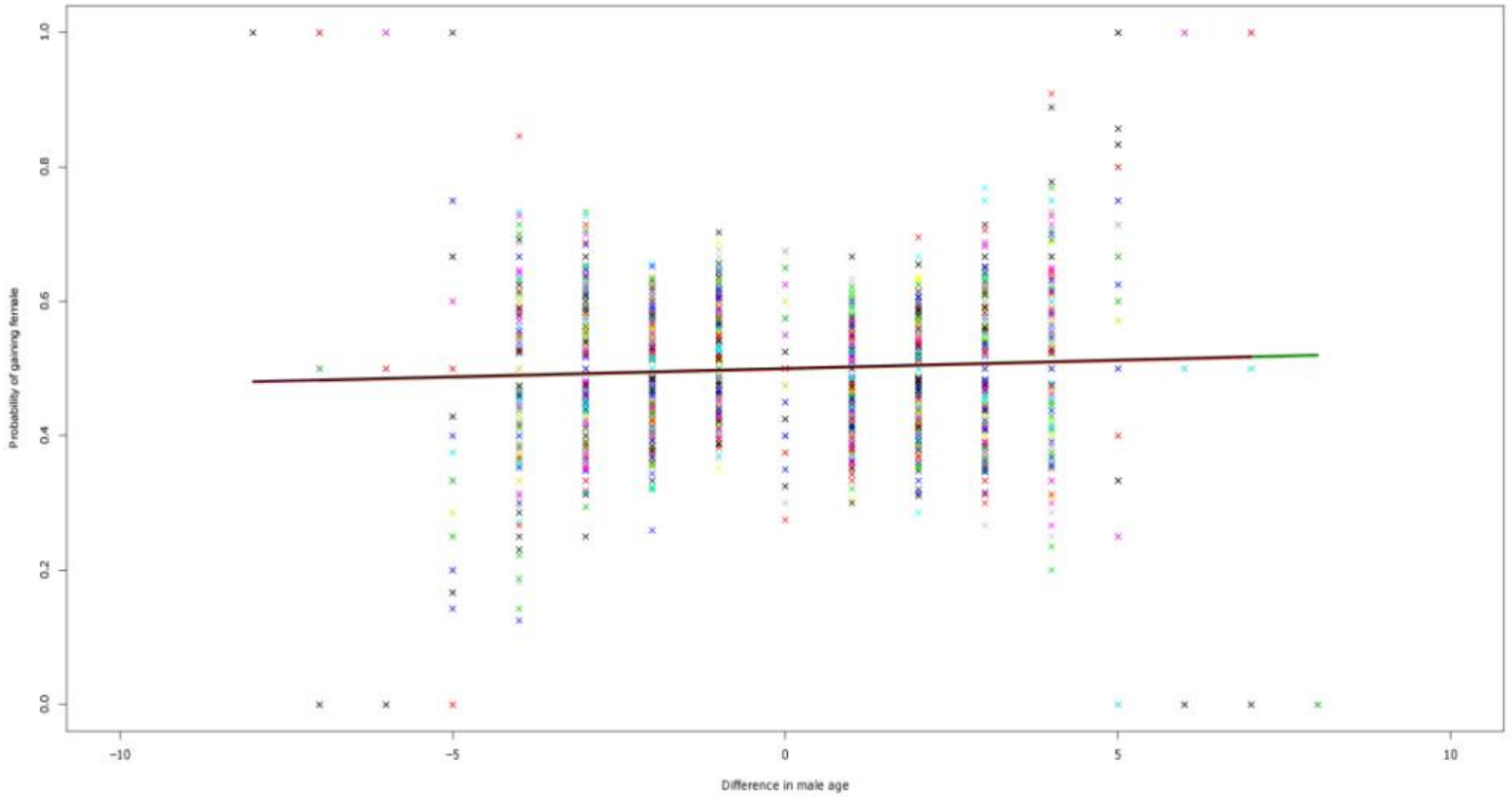


Figure 2.5: the probability that the focal male 'gained' the female against the difference in harem size between the focal and non-focal male: each colour of points and coloured regression line relates to the data generated from one of 1000 samples in which the focal male was randomly selected from the pair of males to be compared (see text). Very large differences in harem sizes outside the 5th and 95th percentile (-15 to +14) are excluded, as these very rare events were subject to large amounts of scatter in the simulations.

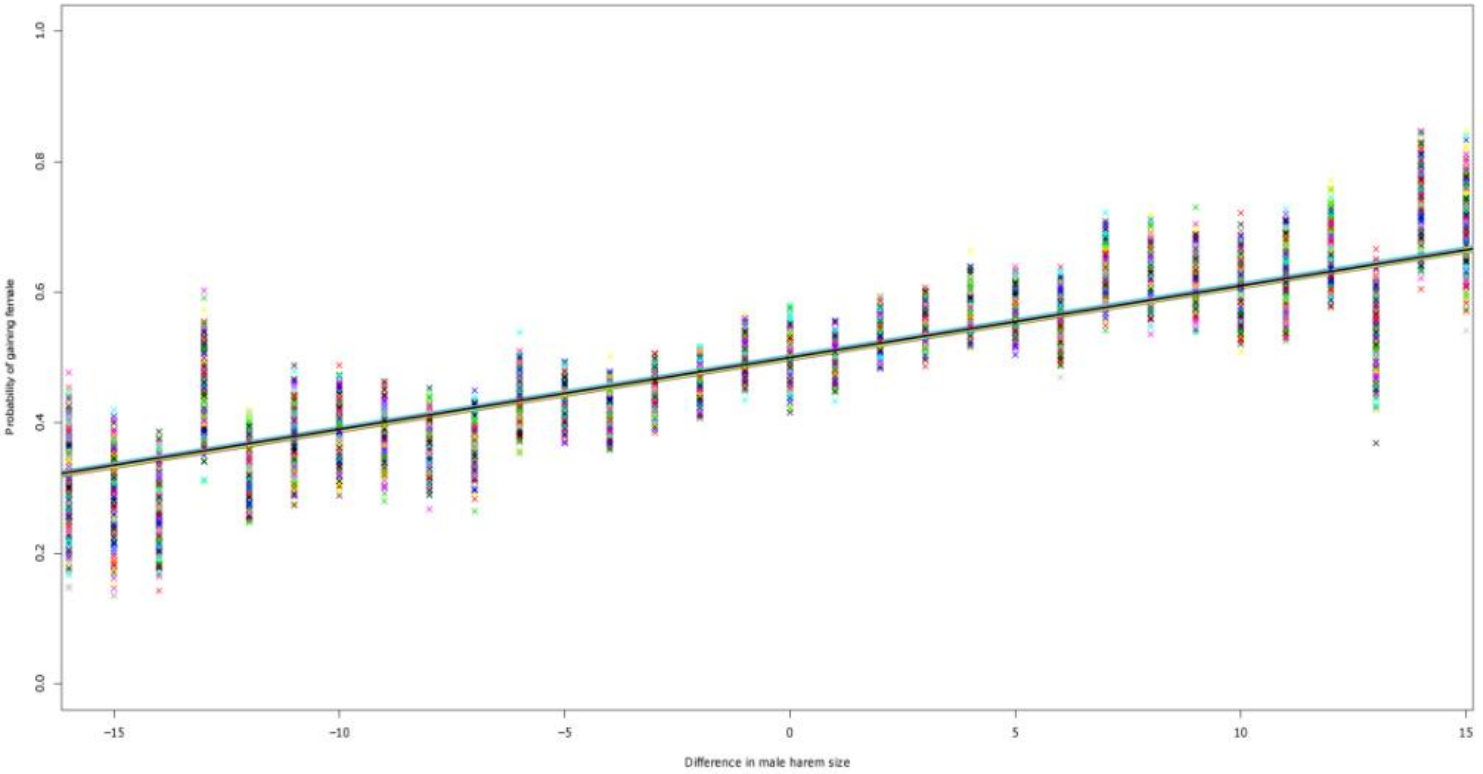


Table 2.3a: results of GLMM, testing the main effects of differences in male phenotype on the probability of a female moving between pairs of males. Results reported here are average and fifth and ninety-fifth percentiles, for estimates, standard errors, Wald and p values from Wald tests for differences in male traits and relatedness to the focal female, for 1000 runs of the GLMM.

Table 2.3b: results of a GLMM, testing interactions between day relative to oestrus and effect of differences in male phenotype on the probability of a female moving between pairs of males with all male traits in the model. Results reported here are average and fifth and ninety-fifth percentiles, for estimates, standard errors, Wald and p values from Wald tests for the interaction between differences in male traits and relatedness to the focal female and day relative to female oestrus, for 1000 runs of the GLMM.

Term	Estimate			SE			Wald value			p value		
	Av.	0.05	0.95	Av.	0.05	0.95	Av.	0.05	0.95	Av.	0.05	0.95
Difference in age	-0.325	-0.351	-0.302	0.034	0.032	0.035	94.054	82.453	106.122	<0.001	<0.001	<0.001
Difference in harem size	0.104	0.098	0.110	0.009	0.009	0.010	122.104	112.041	132.971	<0.001	<0.001	<0.001
Difference in relatedness	2.550	-1.277	3.892	2.611	2.511	2.722	1.045	0.235	2.182	0.350	0.140	0.628

Interaction term	Wald value			p value		
	Av.	0.05	0.95	Av.	0.05	0.95
Day relative to oestrus x difference in age	2.394	0.907	4.277	0.155	0.039	0.341
Day relative to oestrus x difference in harem size	0.143	0.001	0.512	0.768	0.474	0.978
Day relative to oestrus x difference in relatedness	0.181	0.006	0.587	0.718	0.444	0.939

Neither differences between males in their age or harem size, nor differences in their relatedness to the focal female, had an effect on the distance which females moved when changing harem: after simplification, this model retained no main effects of these terms, nor any interactions between these terms and a female's oestrus state (interactions with day relative to oestrus in full model: difference in male age: $F_{1,3929.4}$=1.05,p=0.31, difference in harem size: $F_{1,4606.8}$=0.02, p=0.90, difference in relatedness: $F_{1,4391.6}$=0.01, p=0.90, see table 2.4). This indicates that male phenotype and relatedness to the female had no effect on the distance moved by either oestrous or non-oestrous females.

Table 2.4: F values and p values, based on type II sums of squares, for the full LMM testing for an effect of differences between males in phenotype or relatedness to the female on the distance moved by females when changing harem.

Variable	F value	d.f.	p value
Confidence	0.44	2,2583.8	0.645
Year	1.43	28,3114.2	0.065
Days between observations	19.75	1,4927.6	<0.001
Peak rut	0.04	1,4819.7	0.850
Minimum temperature	5.03	1,4839.7	0.025
Rain	2.46	1,4893.4	0.117
Day relative to oestrus x difference in age	1.05	1,3929.4	0.306
Day relative to oestrus x difference in harem size	0.02	1,4606.8	0.899
Day relative to oestrus x difference in relatedness to female	0.01	1,4391.6	0.903

2.4 Discussion

In this study we have shown that the red deer rut, a famous and well-studied example of male competition, is a more complex and dynamic process than previously appreciated. Changes in harem membership are common, with at least a third of females being found in different harems on consecutive observations. Moreover, changes in harem membership increase when females come into oestrus, a finding in agreement with previous studies of this population, conducted early in the study period (Clutton-Brock *et al.* 1982). In this study we have also demonstrated that when changing harem, oestrous females move substantial distances, up to 4km, to locations outside of their normal rut home ranges. These distances are further than females changing harem at other times in the rut. That females leave their home ranges during these oestrus movements indicates that these behaviours are deviations from normal spatial behaviour. Moreover, such excursions are clearly tightly linked to timing of mating, given that on the day after oestrus, females were

not more likely to be outside their home range than at other times in the rut. Further, around 45% of oestrus excursions result in the male into whose harem a female moved gaining paternity of the female's offspring. This indicates that female excursions have the potential to affect individual male reproductive success and so, potentially, sexual selection on male traits.

Excursions by breeding females, such as those described here, have been noted in other polygynous mammals, but the causes and significance of the behaviour are often poorly understood, or controversial (Richard *et al.* 2008, Hoffman *et al.* 2007, Kotiaho *et al.* 2008b). We predicted that females in oestrus move between harems in order to mate with males which can provide them with direct or indirect fitness benefits, and as such, that female excursions would be non-random with respect to male phenotype. Further, given such movements are likely to be costly (Byers *et al.* 2005), we expected females to accept greater costs, *i.e.* to move further, when the fitness benefits were higher: *i.e.* when the magnitude of the difference in phenotype between the male that the female left and the novel male was greater (*sensu* Hoffman *et al.* 2007, although note this study considered absolute rather than relative male phenotypes). However, although in general females moving between harems did so non-randomly with respect to male phenotype, we found no evidence of preferential movements to harems held by older males, larger harems, or harems held by less related males which were specific to oestrous females; this is in contrast to our prediction. We also found no evidence females moved further when the magnitude of the difference between the male left and the male joined was larger, suggesting females do not move further when the potential benefits are greater. However, it should be noted that the interpretation of this latter result is dependent upon the spatial distribution of males with respect to trait values: if for example, males rutting in similar locations had similar trait values, then females moving further would inevitably move to more dissimilar males.

Our results do show that, throughout the rut, when females changed harem they were more likely to enter the harems of younger males (see figure 2.4a). That said, when male age was considered without correcting for female tendency to join larger harems, we found females in oestrus did not show a tendency to preferentially join older or younger males (figure 2.4b). In general our results provide little evidence that females have preferences for mating with younger males. Female choice for mating with younger males is not generally predicted by theory (although females may prefer young males if, for example, viability/fertility in old age is negatively genetically correlated with early adult

viability/fertility, Trivers 1972, Brooks and Kemp 2001, Hansen and Price 1995), and there is no reason to expect younger males to be better able to protect females from harassment. The effect of male age on female harem changing therefore seems less likely to be the result of female mate preference than an artefact of attempts by young males to segregate small groups of females from harems during fights between more dominant males, or of females having to cross the peripheral harems of young males to move between feeding sites (Clutton-Brock *et al.* 1982). Clutton-Brock *et al.* (1982) found females were less likely to mate with younger males and that the potential costs of mating with inexperienced males were high in terms of injuries gained when mating. Our results hint that females may avoid young males when in oestrus, given the presence of a weak trend for an oestrous female to be less likely to be found with young males after changing harem than females not in oestrus (figure 2.4b).

Secondly, throughout the rut, when females changed harem they were also more likely to be found in larger harems independent of the effect of male age; however again this was not specific to, nor changed when females were in oestrus. Females may have preferences for larger harems because harem size is an indicator of male quality (mediated through male-male competition, Clutton-Brock *et al.* 1979), or because of a tendency to join other females, as has been described in lekking ungulates (Clutton-Brock & McComb 1993). Females may benefit from reduced per capita rates of harassment in larger harems (Clutton-Brock *et al.* 1993, Carranza and Valencia 1999). Alternatively, given male harem size is linked to fighting ability, males with large harems may be more likely to obtain females through male-male competition (Clutton-Brock *et al.* 1982). However, given the tendency of females to be found in larger harems after changing harem was not specific to oestrous females, our findings do not indicate positive evidence that movements of females when in oestrus are a result of female preferences for mating with males holding larger harems.

Overall therefore, although we show movements of females in this population are clearly associated with the timing of mating, and frequently result in mating with the novel male, our results do not support the prediction that females in oestrus systematically move between harems in order to mate with preferred males. Of course, it is important to note that females may show preferences for traits other than those considered here: for example, various studies have suggested female preferences for properties of the male roar (Charlton *et al.* 2007a, Charlton *et al.* 2007b, McComb 1991, Reby *et al.* 2001, Reby & McComb 2003, Reby *et al.* 2010 but see Charlton *et al.* 2008). It is also worth

considering that the optimal mate may not be the same male for all females: for example, females may trade off mating with a male which can provide additive genetic benefits with mating with males which are more genetically compatible (Mays and Hill 2004, Neff and Pitcher 2005); particularly if the optimal balance varies depending on the likely sex of their offspring (Carranza *et al.* 2009). Alternatively, females may vary in their ability to exert mate preferences with condition or experience (Jennions and Petrie 2007). Such variation may mask female preferences for male phenotypes in our study. Further, given females are highly philopatric, and unpublished data suggests males show some consistency in rutting location between years (see Chapter 5), it is possible females do not move to a preferred male, but to a 'different' male; one with whom they had not mated in previous years. Such behaviour could be favoured either to maximise diversity of paternal genetic combinations (Worthington-Wilmer 2001), or because of knowledge of reproductive failure in previous breeding attempts with that male, analogous to 'divorce' observed in monogamous birds (reviewed in Choudhury 1995). However, in general, it appears most likely that much of the harem change we observe amongst females results from passive responses to male disturbance (fights, harassment and so on), rather than preferences for male phenotypic traits, and the reasons for the sometimes extraordinary distances moved by females when in oestrus remain unclear. In contrast to other polygynous mammals (*e.g.* roe deer, Lovari *et al.* 2008), in the harem-defence system of red deer, where males defend groups of females at close proximity rather than territories, females are unlikely to have to search for a mate. Further, although we have found effects of rain and temperature on the distances females move each day, after accounting for these effects females in oestrus still moved significantly further than females not in oestrus. Therefore these excursions are unlikely to be a result of environmental effects (*cf* Twiss *et al.* 2007). Further work is therefore needed to unravel why females in oestrus make such long distance movements, and particularly to explore the link between rates of male disturbance within the harems of different males and both the propensity of females to change harems and the distances travelled by females when disturbed.

Understanding the ultimate causes and consequences of excursions of breeding females in polygynous mammals requires data to link excursions with reproductive state of females, to compare male phenotypes and to assess whether excursions affect which males gain paternity of a female's offspring. Using a uniquely detailed long-term behavioural data we have identified unusual movements of red deer females linked specifically to oestrus, and have shown that these movements have consequences for the reproductive success of individual males. However, the harem changing and movements we observe in oestrous

females appear to be random with respect to male phenotype, suggesting these are passive responses to male-male competition and turnover; yet if this is true, it remains unclear why females move such long distances in their response. It is therefore clear that excursions of females should not be assumed to be expression of female mate choice. However, there remains potential for such movements to affect male mating success, as demonstrated by the number of paternities resulting from female movements in this study. If the causes of movement can be understood, the excursions of female polygynous mammals are therefore likely to be a fertile area for future research in understanding conflict between female and male mating behaviour in such systems.

Chapter 3:

The Impact of Ecology on Sexual Selection

3.1 Summary

Ecological factors, such as sex ratio, the spatio-temporal distribution of mates, and climate, can have important effects on variance in mating success, and so potentially the strength of sexual selection. Although a growing number of empirical studies have found relationships between ecological parameters and metrics of the potential strength of sexual selection, such as standardized variance in mating success, or 'opportunity for sexual selection', I_s, few studies have addressed the interactions between multiple ecological variables on such metrics or, indeed, tested how well they capture the actual strength of sexual selection in natural populations. In this study we investigate the effects of demography, temporal variation in female availability and climate, on inter-annual variation in I_s over a 35 year period in a wild population of red deer. Further, we test whether the same ecological parameters also correlate with annual selection differentials on male secondary sexual traits. We find that the interaction between intensity of competition from males immigrant to the population and temporal availability of females predicts I_s at the population level, and also affects the likelihood of males gaining reproductive success at the individual level. To our knowledge, this is the first evidence that the impact of male density on opportunity for sexual selection in a wild mammal varies with temporal variation in female receptivity. Under low competition the harem defence system of red deer means that variation in female oestrus dates benefits dominant males; but when competition increases the collapse of the mating system towards scramble competition results in variable oestrus dates widening breeding opportunities across males. We found no evidence however that this interaction correlates with selection differentials on rut end date or antler mass, and indeed, I_s correlated poorly with selection differentials on these traits. We did find a trend towards the interaction between male competition and variation in oestrus date correlating with annual selection differentials on rut start dates, suggesting these parameters potentially affect selection on one of the male secondary sexual traits considered. This study therefore empirically demonstrates some utility, but also substantial limitations, to using I_s as a measure of the potential for sexual selection.

3.2 Introduction

Ecological effects are important in shaping sexual selection, influencing the evolutionary dynamics of sex-specific morphological and behavioural traits (Shuster and Wade 2003, Lande 1980). Between species, ecological effects are known to determine mating systems and account for variation in sexual dimorphism (Emlen and Oring 1977, Clutton-Brock and Harvey 1978). Within species, changes in the ecological factors constraining sexual selection can result in changes in the magnitude or direction of sexual selection under different environmental conditions, between seasons and populations (Kokko and Rankin 2006, Cockburn *et al.* 2008, McLain *et al.* 1993, Punzalan *et al.* 2010). Further, the magnitude and direction of the effects of ecological factors on sexual selection is expected to differ between populations (Emlen and Oring 1977, Kokko and Rankin 2006), and may depend on the interaction between multiple factors (Kokko and Rankin 2006, Ims 1988). In this study, we examine the effects of demography, variance in female oestrus date and climate on inter-annual variation in the opportunity for sexual selection in a population of wild red deer over a 35 year study period.

The potential for sexual selection arises from competition amongst individuals of one sex (generally males in polygynous species) for access to breeding opportunities, resulting in a non-random distribution of mating success amongst individuals. Sexual selection theory predicts that the 'Operational Sex Ratio', the ratio of ready to mate males to receptive females in the population, will have important effects on the intensity of this competition as it determines the rate at which individuals encounter potential mates and competitors (OSR, Emlen and Oring 1977, Clutton-Brock and Parker 1992); although recent research has also indicated the importance of mate encounter rates and costs of breeding (Kokko and Monaghan 2001, Kokko and Johnstone 2002, Fitze and Le Gaillard 2008). Demographic population parameters, *i.e.* population density and sex ratio, are key determinants of the Operational Sex Ratio (Clutton-Brock and Parker 1992, Kvarnemo and Ahnesjö 1996, Jirotkul 2000, Clutton-Brock *et al* 1997, Eshel 1979, McClain *et al.* 1993, Fleming and Gross 1994). However, the consequences of demographic parameters on the opportunity for sexual selection are frequently variable (Emlen and Oring 1977, Kokko and Rankin 2006, McLain 1992, Reichard 2004a, Jirotkul 1999), and in general, the effect of OSR on sexual selection may vary between and within populations if other ecological parameters affect the ability of males to monopolize access to females (Emlen and Oring 1977, Ims 1988, Shuster and Wade 2003, Shuster 2009, Klug *et al.* 2010a). For

example, even where OSR is even, the potential for sexual selection will increase if individual males can successfully monopolize multiple females (Ims 1988). However, to date we do not know how mate monopolizability affects the opportunity for sexual selection across changing OSRs (Klug *et al.* 2010a).

Empirical studies of ecological effects on sexual selection frequently use metrics based on intra-sexual variation in mating success as a metric of the strength of sexual selection (Shuster and Wade 2003, Mills *et al.* 2007a, Jones 2009, Klug *et al.* 2010a). Standardized variation in mating success sets the upper limit for changes in phenotype caused by variation in mating success, and is known as the 'opportunity for sexual selection' or I_s (Crow 1958, Arnold and Wade 1984). I_s has been used to examine effects of sex ratio, environmental change and climate on sexual selection (Jirotkul 2000, Weatherhead 2008, Järvenpää and Lindstrom 2004, Perlut *et al.* 2008, Twiss *et al.* 2007). However, I_s confounds the possibility for evolutionary change due to selection and that due to drift following random mating (*e.g.* Koenig and Albano 1986, Grafen 1987, Andersson 1994a), and a recent theoretical paper has shown that, in simulations, I_s and the actual strength of sexual selection are only correlated under restrictive conditions (Klug *et al.* 2010a). In particular, Klug *et al.* point out that changes in I_s with variation in OSR can be unrelated to the strength of sexual selection unless the degree to which males with high trait values can monopolize females also changes as the OSR becomes male biased. Therefore, when using I_s to measure the intensity of sexual selection, it is appropriate to assess how the measure is correlated with annual selection differentials on traits that affect male competitive success: in this study therefore we consider both ecological effects on opportunity for selection and whether the same effects also correlate with annual selection differentials on male secondary sexual traits.

Various ecological factors have been postulated to cause changes in the ability of males to monopolize access to females, including the relative competitive abilities of males, the spatio-temporal distribution of receptive females, and the influence of climate. The relative competitive abilities of individuals can vary with population age structure, which is particularly relevant if the effects of population density and sex ratio on mating success are age-dependent (as shown in male reindeer, Mysterud *et al.* 2003, and common lizards, Dreiss *et al.* 2010). The success of alternative male tactics is often dependent on ecological factors: for example, in bitterling, sneaking behaviour increases with male density (Reichard *et al.* 2004a). In ungulates, the reproductive behaviour of sub-adult

males frequently depends on the sex ratio and age structure of a population (bison, Komers *et al.* 1994, mountain sheep, Singer and Zeigenfuss 2002, Soay sheep, Stevenson and Bancroft 1995, reindeer, Mysterud *et al.* 2003) and this can affect the role of sex ratio and density in determining the intensity of competition (Bonenfant *et al.* 2004). The effect of the spatio-temporal distribution of receptive females on the ability of males to control access to females has long been a topic of interest in theoretical literature (Emlen and Oring 1977, Ims 1998, Shuster and Wade 2003, Clutton-Brock and Parker 1992). Low levels of reproductive synchrony and low levels of spatial clustering of females are predicted to allow dominant males to monopolize fertilizations, resulting in high variance in male mating success. In contrast, a dispersed distribution of females and high levels of reproductive synchrony are likely to prevent dominant males from controlling all sexually receptive females because of the required 'handling time' per female (Emlen and Oring 1977, Ims 1988). In both feral cats and the butterfly, *Heliconius charitonia,* variance in male reproductive success has been shown to be higher when females breed asynchronously (Say *et al.* 2001, Mendoza-Cuenca and Macias-Ordonez 2009).

Finally, climate can also affect males' ability to defend receptive females (*e.g.* Twiss 2007). In general, in recent years there has been interest in the impact of climatic variables on sexual selection (Twiss *et al.* 2007, Møller and Szép 2005, West and Packer 2002, Weatherhead 2008, Isaac 2009); many studies have demonstrated changes in breeding phenology linked to climate change in birds and mammals (Reale *et al*. 2003, Post and Forchhammer 2008, Both and Visser 2001, Crick and Sparks 1999, Thackeray *et al.* 2010 and see Sparks and Menzel 2002), and there is also now evidence that climate can directly impact selection on sexual traits (Møller and Szép 2005), and potentially affect variance in mating success (Isaac 2009). Climatic changes outwith the breeding season can result in differential mortality between the sexes, and therefore a change in the operational sex ratio, with implications for *Is*; this has been shown in red winged blackbirds (Weatherhead 2008). Climate also potentially affects the strength of selection through impacts on condition: in superb fairy wrens, selection on a sexual trait (early moulting) is stronger when males are in good condition following favourable weather conditions (Cockburn *et al.* 2008). Finally, climate may affect the distribution of paternities during the breeding season through changes in sexual behaviour. Twiss *et al* (2007) investigated the impact of climate during the breeding season on the opportunity for sexual selection in a population of grey seals, and found a positive correlation between opportunity for sexual selection and rainfall.

However, despite numerous such studies empirically testing the effects of particular ecological parameters on sexual selection, to date the interaction between the variables which can affect male ability to control females and other ecological parameters, such as population density and sex ratio, has received little attention in wild populations.

The present study

Here we consider how ecological variables affect the standardized variance in mating success, I_s, in a population of wild red deer living in the North Block of the Isle of Rum. The red deer mating system is characterized by harem-defence polygyny with high, but variable, levels of skew in male mating success (Clutton-Brock *et al.* 1982). The population was released from culling in 1972, after which it grew steadily until the early 1980s, when it stabilized at carrying capacity. This rapid population growth was associated with male-biased juvenile mortality and increased male-biased dispersal, resulting in a female-biased population sex ratio (Clutton-Brock *et al.* 1997). In a previous study Clutton-Brock *et al.* (1997) addressed the impact of these changes on skew in male reproductive success. The authors found that as population density increased, the population sex ratio (assessed outside the breeding season) became increasingly female-biased, and this was associated with a reduction in the average age of harem-holding males and an increase in the proportion of males holding harems, suggesting a decline in the intensity of competition for mates. However, their study was based on behavioural measures of reproductive success; we are now able to assess reproductive success genetically. Although behavioural and genetic paternities are highly correlated in the study population, behavioural estimates of paternity have been shown to underestimate variance in male breeding success (Pemberton *et al.* 1992). Further, it should be noted that in this population, sex ratio outside the breeding season may differ from sex ratio during the rut itself, as males are not generally resident in the population and instead immigrate into the study area during the rutting period. In addition, not all females in this population conceive each year, and so the number of females in oestrus, and so 'ready to mate' may be a more direct measure of female availability than total females. In general the effects of demographic parameters directly influencing OSR during the breeding season remain to be investigated.

In addition, we predict an effect of many potential ecological parameters further to those considered by Clutton-Brock *et al.* on the ability of males to monopolize females in this

population. Firstly, females come into oestrus over an approximately two month period: theory predicts that as the synchrony of female oestrus increases, variance in male reproductive success will decrease (Ims 1988). In Norwegian red deer, female oestrus was later and more synchronous at higher population densities (Mysterud *et al.* 2008); further, the synchrony of female oestrus is also likely to be affected by female age structure, as younger females ovulate later than prime aged females (Langvatn *et al.* 2004). Secondly, there is potential for the relative competitive abilities of males to vary, firstly with age structure, but also with the number of males rutting which were not born to the study area. Previous work suggests immigrant males are stronger competitors than those males born to the study area (Clutton-Brock *et al.* 1997, Clutton-Brock *et al.* 2002). We therefore expect an increase in competition, and so an increase in variance in male reproductive success when i) the age structure of the population is less biased towards young males and ii) more immigrant males are rutting. Thirdly, we also consider the effects of rainfall and temperature on I_s in this population, as sexual behaviour may be constrained by conditions during the rut. For example, rainfall during the rut has been found to be associated with higher levels of female movement between harems; this could undermine the outcome of male competition on harem holding and so decrease variance in male reproductive success (see Chapter 2).

Our initial population-level analyses of these variables show that I_s is determined by an interaction between the number of immigrant males rutting and the variance in female oestrus date (see results). To understand the underlying causes of this effect, we examine how these parameters affect male breeding success at the individual level. We therefore test the hypothesis that higher numbers of immigrant males rutting depress the breeding success of relatively unsuccessful males at the individual level, and that the outcome of this competition is affected by the variance in female oestrus date. Finally, we aim to determine whether the ecological impacts that affect variation in mating success translate into impacts on actual sexual selection: to do this we test whether the same ecological effects that affect I_s are also correlated with annual selection differentials on male traits. We consider selection differentials with respect to standardized rut start date, rut end date and antler mass. Antler size has been previously shown to be under selection (Kruuk *et al.* 2002b). Early rut start dates have also been associated with average annual breeding success (Moyes *et al.* 2010). Rut end dates have recently been shown to be advancing twice as fast as rut start dates in response to local climate warming (Moyes *et al.* 2010); it

is therefore interesting to examine how ecological parameters influence selection on this trait.

3.2 Methods

Study System

All data was collected from a long term study of a free-living red deer population living in the North Block of the Isle of Rum, off the west coast of Scotland. In this population, all individuals of both sexes can be recognized, either from individual idiosyncrasies or from artificial marks (Clutton-Brock *et al.* 1982). Individual life histories have been closely monitored in this population since 1971 (see Clutton-Brock *et al* 1982); in this analysis we use data from ruts 1971-2006, but exclude 1973, for which little rut data is available. We also repeat our analyses using data from 1982 onwards only, to investigate whether ecological influences on I_s differed after the population reached carrying capacity (Clutton-Brock *et al.* 1997, see section 3.1).

Females in the population usually remain within the study area throughout the year, whilst adult males born to the population spend the majority of the year outside the study area, returning for the rut. The male population during the rut includes both natal males born in the study area and between 9 and 31 non-natal males, who were born outside the study population (hereafter called immigrant males). Both natal and immigrant males reside outside the study area outside of the rut, and temporarily immigrate into the study area (back into the area for natal males) during the rutting period.

Population size data is taken from censuses conducted five times each month between January and May, when the number and location of all individuals seen is recorded. During the rut (15th September to 15th November), daily censuses are conducted which record all females seen and all males seen with at least one female, providing details on harem size and composition. Females are in oestrus for 24-48 hours and generally mate only once (Clutton-Brock *et al.* 1982, Pemberton *et al.* 1992). Although oestrus dates can be estimated behaviourally, this data is not available for all females due to the brief nature of female oestrus and the size of the study area; instead conception dates can be estimated by backdating from the birth date of subsequent calves by the estimated gestation period of 235 days (Clutton-Brock *et al.* 1982).

Variables Used

Standardized variance in male mating success (I_s): for all males seen with a female during a rut, *i.e.* all 'rutting males', annual breeding success (ABS) was calculated from the pedigree. To this, we added paternities assigned to "dummy males" (see pedigree description below), and calculated I_s as the variance in annual breeding success divided by the mean annual breeding success squared.

Rut sex ratio: the number of females aged two and above present in rut censuses, divided by the number of rutting males (see below). Females are first able to conceive at the age of two in the study population; therefore this variable is the ratio of sexually mature females to males. By using females/male, this measure directly reflects the potential mating opportunities for each male, all else being equal.

Population Size: the number of adult females (aged 1 and above) present in more than 10% of censuses between January and May (Coulson *et al.* 1997).

Number of rutting males: the number of males which were seen with at least one female in at least one census during a particular rut..There are a median of 11 males holding harems each day of the rut, ranging from 1 to 27 unique males holding a harem on any day.

Number of immigrant males rutting: the number of non-natal males which were seen with at least one female in at least one census during a rut.

Number of oestrous females: the number of females present in rut censuses which went on to produce a calf in the following calving season.

Average age of rutting males: the average age of all males which were seen with at least one female in at least one census during a particular rut.

Variance in oestrus date: approximate oestrus date was calculated for each female by backdating from the birth of her calf by 235 days as above (Clutton-Brock *et al.* 1982). We then adjusted these dates by calculating the day within an eleven day period surrounding this date (the 'oestrus window', Clutton-Brock *et al.* 1982) which was closest to the day on which the female was seen with the male that was assigned the paternity of her calf. We then calculated variance in these adjusted dates for each breeding season and used this as the variance in oestrus date for each year. This results in a small reduction in

sample size of oestrous females where paternities are not assigned, but given we are interested in variance in dates over a relatively short time period, the accuracy provided by this calculation was more important than the loss of sample size.

In studies of avian mating systems, explicit measures of female synchrony (*i.e.* the percentage of females likely to be fertile on the same day) have been developed to investigate the effects of breeding synchrony on, for example, the rate of extra pair paternity (Kempenaers 1993). However, in this population of red deer, on average only 1.74 females are in oestrus on the same day, increasing to a maximum of 7 during the peak of the rut. Therefore, female synchrony within particular days is unlikely to influence the ability of males to defend oestrus females; rather, the effect of variance in female oestrus date is more likely to result from the spread of oestrus dates around the mean date (and therefore male stamina in continuing to rut over this spread), which is better captured by the measure of variance rather than a measure of synchrony on any day.

Maximum temperature: the average daily maximum temperature between the 15th September and the 15th November. This is recorded on Rum by Scottish Natural Heritage and available from the Met Office British Atmospheric Data Centre. However, there are frequent missing records: to estimate these values, temperatures on Rum were predicted using a regression equation from those on Tiree, a nearby island with more complete climate records, with which Rum temperatures are highly correlated (R=0.971, Moyes *et al.* 2010). Where gaps remained, temperatures were estimated from the mean temperature over the previous three days and subsequent three days (Moyes *et al.* 2010).

Rainfall: average daily rainfall between the 15th September and the 15th November. Again, rainfall is recorded on Rum by Scottish Natural Heritage and available from the Met Office British Atmospheric Data Centre, but there are missing records. In this case, a regression equation was used to predict the rainfall from records taken at Rhubana, Inverness-Shire (Moyes *et al.* 2010), with which Rum rainfall is again highly correlated (R=0.909).

Rut start date: the first day on which a male rutted (held a harem of at least one female) in any rut, restricted to males who rutted for at least five consecutive days in any rut, in Julian days since 1st January.

Rut end date: the last day on which a male rutted in each rut, restricted to males who rutted for at least five consecutive days in any rut, in Julian days since 1st January.

Antler mass: mass of cast antlers (grams) found in the spring following each rut: where both antlers of a male are found, an average mass was taken. Antler mass records were available for 220 males, totalling 524 records.

Paternity assignment

In the calving season, the majority (approximately 80%) of calves are caught and, since 1982, tissue samples taken for genetic analysis (Marshall 1998, Pemberton *et al.* 1992). The majority of long lived animals born prior to this date have been genotyped retrospectively, from samples taken from carcasses, cast antlers or animals when darted. Individuals born since 1991 are genotyped at 15 highly variable microsatellites; prior to this individuals are genotyped at 8 microsatellites. In the dataset used in this study, 58% of calves are assigned a father (including 'dummy fathers', see below) with greater than 80% individual confidence (Walling *et al*. 2010). Paternities were assigned using the programs MasterBayes (Hadfield *et al.* 2006) and COLONY2 (Wang and Santure 2009). MasterBayes assignments were used preferentially, unless MasterBayes did not assign a father, in which case COLONY2 was used. Details of paternity assignment are given in Walling *et al.* (2010), but two points are salient to this study: firstly, in COLONY2, genetic assignments are supplemented by identification of clusters of half sibs. Where half sibs could not be assigned a father, a dummy male was assigned to maintain that half-sib group and the associated annual breeding success: we did this because identifying breeding success of all males which rutted, even those that are unknown, is important for the calculation of variance in male mating success. Secondly, in MasterBayes, genetic information was supplemented by phenotypic information on known predictors of paternity: age and the number of days on which a male was seen with a female during her oestrus window (Clutton-Brock *et al.* 1982, Pemberton *et al.* 1992, Kruuk *et al.* 2002b).

Using phenotypic information (*i.e.* age), and behavioural information about harem-holding in our analyses of annual breeding success presents obvious potential circularities. Further, our pedigree is incomplete, and in particular is likely to be biased against assigning paternities to males that are genetically unsampled, particularly when they are young. This is because phenotypic information on harem holding or age used by MasterBayes is unlikely to predict paternity assignments for young males, and so for

young unsampled males there is neither phenotypic nor genetic information with which to assign such males paternities. This bias could potentially create spurious differences in breeding success between young natal and immigrant males, as immigrant males are less likely to be genotyped. Therefore we repeated both the population level analyses, and the individual level analyses of annual breeding success and likelihood of failing to breed detailed below, using a second pedigree in which paternity assignments were generated by MasterBayes (again at 80% individual confidence), but ungenotyped males were excluded from the candidate male population. Further, there has been substantial change in the percentage of calves assigned a father increased over time (effect of year on percentage calves assigned=0.074±0.038, z=1.98, p=0.048), and in particular, it is consistently greater after 1982; by repeating analyses using data from 1982 onwards only (see above), we can test for whether this potentially non-random reduction in paternity data from before 1982 affected results.

Analysis

All individual level analyses of male breeding success were conducted in Genstat v11.1 (VSN International, Hemel Hempsted, UK, Payne *et al.* 2007) and all other analyses were conducted in R 2.8.1 (R Development Core Team 2008).

Factors affecting I_s

I_s was log-transformed to achieve a normal distribution. All demographic variables and climatic variables were initially fitted alone in linear models with I_s as the response variable. We then proceeded to fit multiple explanatory variables within the same model. However, due to the large number of explanatory variables, it was not possible to fit all demographic and climatic variables and interactions in a single model. Therefore, we first fitted a linear model of all demographic variables (rut sex ratio, population size, number of males rutting, number of immigrant males rutting, number of oestrous females, variance in oestrus date and average age of rutting males) with all two way interactions to I_s. The full model was simplified using stepwise deletion, by dropping the least significant term in each model, assessed using F tests with Type II sums of squares *(i.e.* comparing the full model to one with the relevant term removed), and retaining terms if they were significant at the α=0.05 level. To confirm the simplest model was indeed the minimum adequate model, each non-significant term was then returned to the model in turn, and a significant increase in model fit tested for in the same manner. To the minimum adequate

model, we added the climatic variables, maximum temperature and rainfall, and their two way interactions. This model was simplified in the same way. In this way we were able to test the role of climatic variables having taken account of population demography, whilst avoiding over-specifying the model. We also verified that temporal autocorrelation in I_s was not influencing our findings. We did this by comparing the fit of the simplified linear model to one with the same parameters, but in which a continuous autoregressive process correlation structure was also modelled.

Individual level breeding success models

At the population level, we found that an interaction between the number of immigrant males rutting and variance in female oestrus date significantly affected I_s. The aim of our second analysis was to understand the proximate causes of this effect, *i.e.* how this interaction influenced individual breeding success. We therefore tested whether competition from increasing numbers of immigrant males depressed breeding success, and whether this was due to fitness differences between natal and immigrant males; and finally whether the effects of competition on individual breeding success were dependent on female temporal availability. Measures of annual breeding success were available for 603 males, with a total of 2083 records for analysis. We performed three generalized linear mixed effects models with i) annual breeding success (the number of offspring a male sired in each year), ii) failure to breed (1/0, with 1 indicating failure) and iii) rut duration (number of days from when an individual first held a harem to last held a harem during a single rut) as response variables. A 'failure to breed' model was included in addition to annual breeding success as it is potentially more illuminating to selection: if males fail to breed, their genes are more strongly selected against than if they attain a low level of breeding success. Moreover, many males in the population fail to breed entirely whilst those that do breed vary considerably in ABS; therefore by including both measures in our analysis we can gain understanding of what affects each of these components of male reproductive performance. However, it should be noted that as many individuals have zero ABS, ABS as a trait necessarily also reflects some of that described by 'failure to breed'. Rut duration was included to specifically test a hypothesis arising from our population level findings: that increased competition would result in reduced rut durations, as males became exhausted more quickly. We fitted a negative binomial error structure to the model for ABS, binomial for failure to breed, and poisson to the rut duration model. In all models year and male identity were fitted as cross-classified random effects to account for stochastic variation between years and repeated measures on

males. Dummy sires (from the COLONY2 paternity analysis) were not included in this analysis.

As fixed effects we fitted male age, age as a quadratic term, whether a male was immigrant or natal, the number of harem-holding immigrant males and variance in female oestrus date. Two way interactions were fitted appropriate to testing the predictions that i) the effects of competition depend on a male's competitive ability, and ii) the effect of increased competition on individual breeding success is determined by the synchrony of female oestrus date. Specifically, we tested interactions between age (and its quadratic term) with immigrant status and the number of immigrants rutting, the interaction between immigrant status and the number of immigrant males rutting, and the interaction between variance in female oestrus date and the number of immigrant males rutting.

Selection Differentials

To calculate standardized selection differentials for each year, we constructed regression models where the response variable was relative annual breeding success (the individual's ABS divided by the yearly mean ABS), and the explanatory variable was the interaction between either standardized rut start date, rut end date or antler mass (individual trait value divided by standard deviation of yearly trait values), and year, fitted as a categorical value. By doing this, regression coefficients are essentially calculated for each year; as such the coefficients of the models become standardized yearly selection differentials (Lande and Arnold 1983). We then constructed linear models to test i) for correlations between the yearly selection differentials and I_s, and ii) whether the factors determining I_s (the number of immigrant stags rutting and variance in female oestrus date, see below) were also significant predictors of selection differentials.

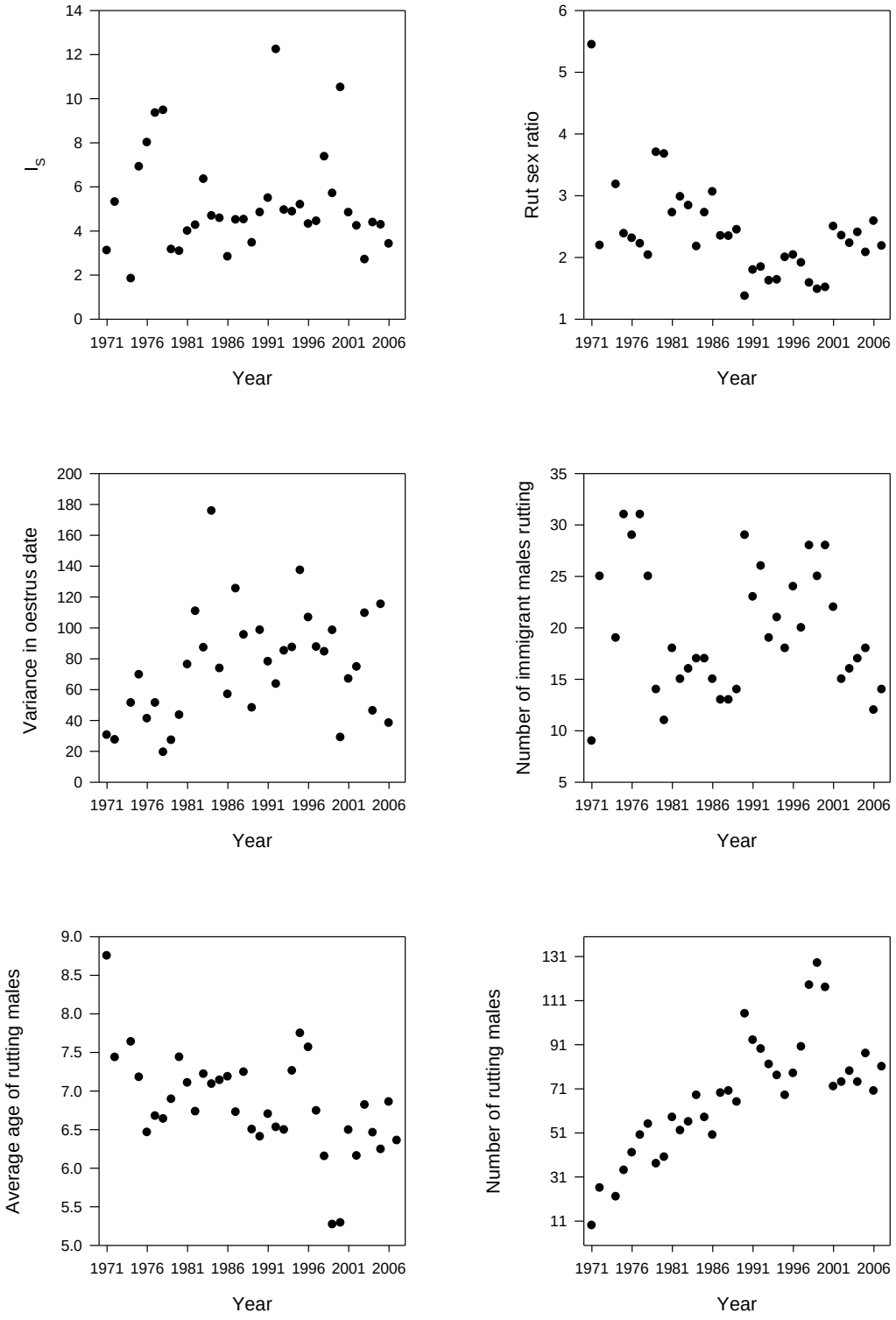
3.4 Results

Ecological factors affecting I_s

There was marked temporal variation in the ecological variables used in our analyses (see figure 3.1). Three measures showed unusual variation in the very early (pre-1975) part of the study: rut sex ratio, number of immigrant males rutting and the average age of rutting males. However, when the dataset was restricted to include only 1975 onwards to exclude these data points, the results were consistent with those reported below using the full dataset (results not shown for reduced, 1975 onwards, dataset). A correlation matrix for

the variables used, excluding I_s, is given in table 3.1. Figure 3.2 shows the distribution of oestrus dates in each year; in years of high variance there is a tail of late oestruses which does not exist in years of low variance in oestrus date.

Figure 3.1: variation in demographic and climatic variables over the time period of the study.



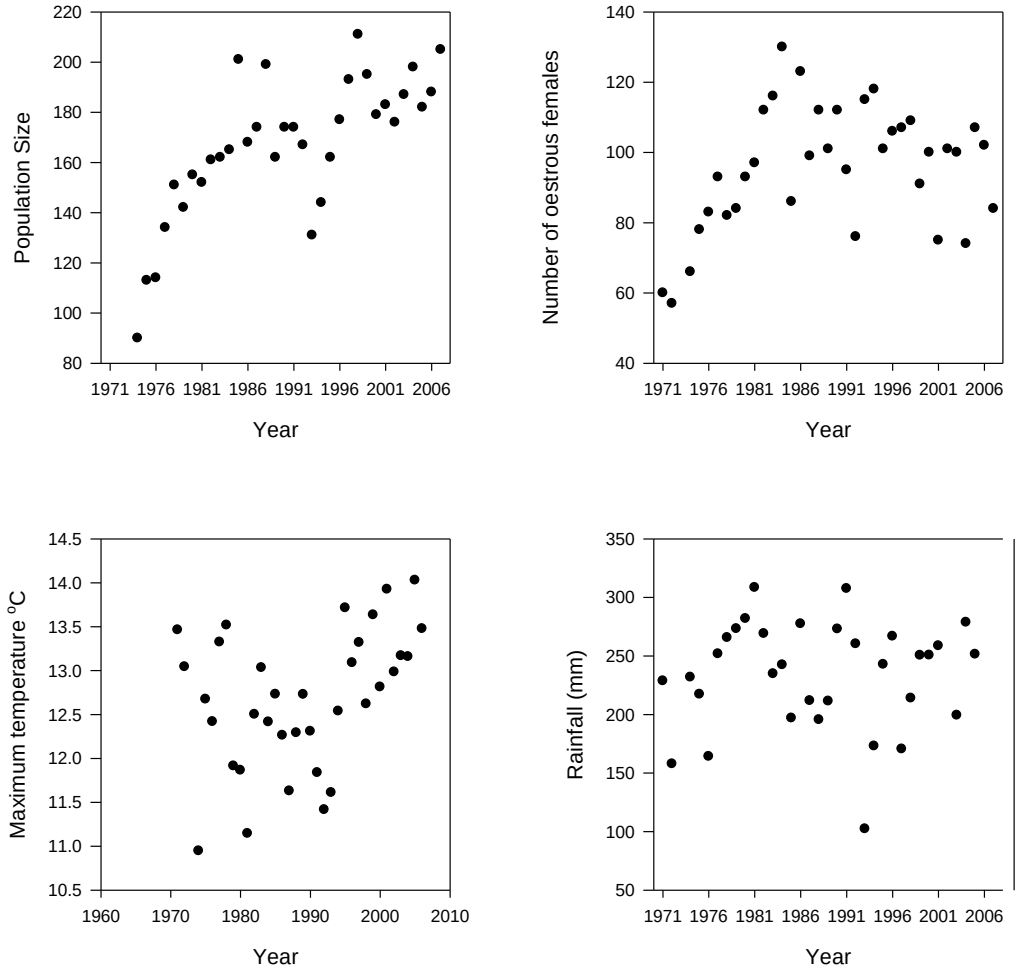


Table 3.1: correlation matrix of demographic and climatic variables used in the population level analysis of I_s.

	Year	RSR	P	RM	IM	OF	VO	AM	MT
Rut sex ratio(RSR)	-0.55								
Population Size (P)	0.71	-0.31							
No. rutting males (RM)	0.78	-0.81	0.68						
No. immigrant males (IM)	-0.01	-0.61	-0.17	0.32					
No. oestrous females (OF)	0.19	-0.24	0.28	0.29	-0.25				
Variance in oestrus date (VO)	0.29	-0.29	0.30	0.28	-0.21	0.58			
Average age rutting males (AM)	-0.08	0.24	0.02	-0.18	-0.38	0.14	0.29		
Maximum temperature (MT)	0.49	-0.30	0.41	0.29	0.20	0.05	0.14	-0.31	
Rainfall (R)	-0.07	0.28	0.14	-0.04	-0.01	-0.19	-0.13	-0.11	-0.01

Figure 3.2: distribution of oestrus dates within each year and variance in oestrus date plotted against year for the time period of the study.

The rut sex ratio and all three measures of male competition - the number of rutting males, the number of immigrant males and the average age of harem-holding males - were significantly correlated with I_s when fitted individually in models of I_s (number of rutting males: Est.=0.004±0.002, $F_{1,33}$=4.81, p=0.04, number of immigrant males: Est.=0.040±0.007, $F_{1,33}$=36.66, p=<0.01, average age of harem-holding males: Est.=-0.210±0.086, $F_{1,33}$=5.97, p=0.02). All other variables were not significantly correlated with I_s (see table 3.2). When the rut sex ratio was more female biased, I_s decreased. As the number of rutting males increased, I_s increased, possibly because of increased competition between males generating an increase in the number of males failing to breed. This was true for both the total number of males and the number of immigrant males rutting; however, the R^2 of the latter model was greater (0.53 compared to 0.13), suggesting that this metric is a better predictor of competition between males. Finally, I_s decreased as the average age of harem-holding males increased.

Table 3.2: results of linear models for the full dataset, where the response variable was I_s (log transformed), and the explanatory variables were as below, each fitted alone. Estimates and standard errors are given, as well as F values, degrees of freedom and p values for comparing the full model to one with the term removed.

Variable	Estimate	S.E.	F value	d.f.	p value
Rut sex ratio	-0.227	0.064	12.64	1,33	<0.01
Population Size	0.000	0.002	0.01	1,31	0.92
No. rutting males	0.004	0.002	4.81	1,33	0.04
No. rutting immigrant males	0.040	0.007	36.66	1,33	<0.01
No. oestrous females	-0.001	0.003	0.09	1,33	0.77
Variance in oestrus date	-0.001	0.002	0.32	1,33	0.57
Average age of rutting males	-0.210	0.086	5.97	1,33	0.02
Average maximum daily temperature	0.061	0.074	0.68	1,33	0.41
Average daily rainfall	0.000	0.001	0.03	1,31	0.85

In the full model including all demographic variables and their interactions, the only significant effect on I_s was an interaction between the number of immigrant males rutting and variance in oestrus date (see table 3.3, figure 3.3a and 3.3b). When the number of

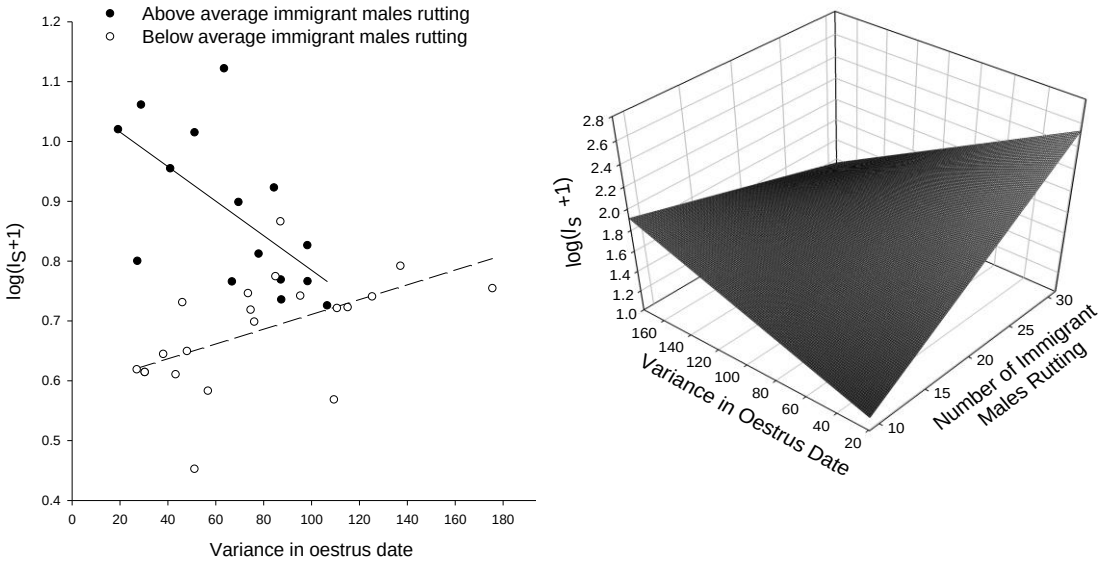
immigrant males rutting was low, I_s increased slightly as female oestruses became more variable; in contrast when the number of immigrant males rutting was high, I_s strongly decreased with increasing variance in female oestrus. The minimum adequate model contained only this interaction: all other terms were non-significant and therefore dropped from the model. To confirm this was the best minimum adequate model, we added, one at a time, each of the main-effects terms which had been dropped in model-simplification back into this final model; on doing this there was no significant improvement in model fit. Further, there were no significant climatic effects on I_s. Maximum temperature and rainfall were added to the minimum adequate model of demographic variables reached above with up to two way interactions, but after simplification only the interaction between the number of immigrant males rutting and variance in oestrus date remained. This result was consistent when repeated using data from 1982 onwards only (interaction between the number of immigrant males rutting and variance in oestrus date: Estimate= -0.0009±0.0002, $F_{1,21}$=12.51, $p<0.01$). Finally, fitting a continuous autoregressive process to account for temporal autocorrelation did not significantly improve the model of the full dataset, indicating that our findings were not confounded by temporal autocorrelation in I_s (phi= 0.146, comparison of models with and without temporal autocorrelation, log likelihood ratio=0.194, p=0.660), nor was there any change in the significance of the immigrant males by variance in oestrus date interaction (p=0.01).

Table 3.3: results of the population-level model, with I_s (log transformed) as the response variable. Only the results of the minimum adequate model are given. For main effects, estimates, standard errors, F values, degrees of freedom and p values are given for comparing the full model to one with the relevant term removed, in a model excluding the interaction (to allow interpretation of the main effects). For the interaction term estimates, standard errors, F values, degrees of freedom and p values are given for comparing the model containing the interaction to one with only main effects.

Variable	Estimate	S.E.	F value	D.F	P value
No interaction					
Intercept	1.00372	0.17061			
No. immigrant males (IM)	0.03983	0.00673	35.01	1,32	<0.01
Variance in oestrus date (VO)	-0.00027	0.00116	0.05	1,32	0.82
With interaction					
Intercept	0.36815	0.28793			
IM*VO	-0.00057	0.00022	6.92	1,31	0.01

Figure 3.3a: effects of variance in oestrus date on I_s, split by the number of immigrant males rutting. The filled circles and solid lines refer to average or greater numbers of immigrant males rutting, open circle and dashed line to lower than average numbers of males rutting.

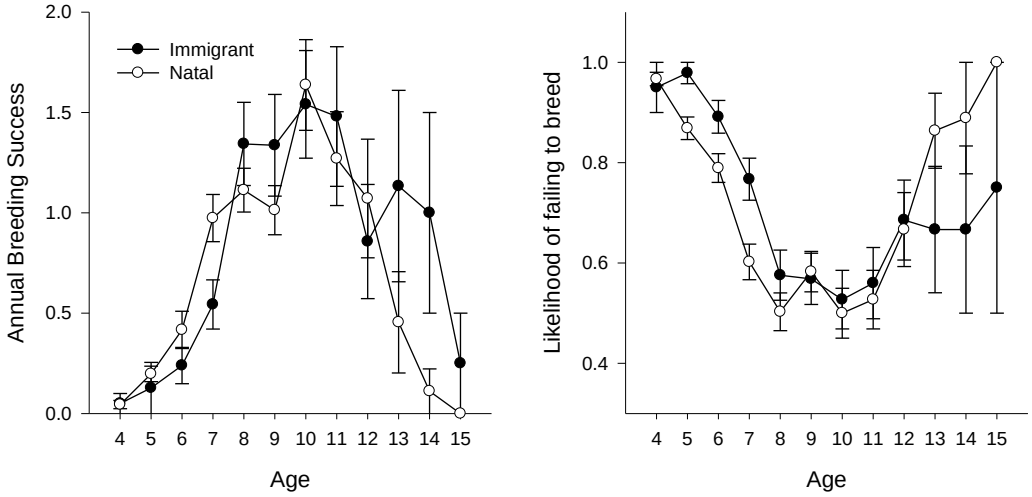
Figure 3.3b: model prediction of interaction between variance in oestrus date and the number of immigrant males rutting on I_s.



Immigrant males had higher annual reproductive performance than natal males. We found a significant interaction between a male's age and whether he was immigrant or natal to the population in models of ABS and likelihood of failing to breed (table 3.4a and 3.4b, figure 3.4a and 3.4b). Immigrant males appeared to have lower annual breeding success and be less likely to breed when young (below 8) but have slightly higher ABS and be more likely to breed when old. However, as described above, it is likely that our pedigree is biased against assigning paternities to young unsampled males, and this could generate spurious differences between young immigrant and natal males. When we repeated the analysis using only sampled individuals, there was no significant interaction between age and immigrant status on ABS; instead, immigrant males had significantly higher ABS (figure 3.5a, effect of being natal on ABS: -0.4952±0.1543, $F_{1,277.5}$=8.85, p<0.01, table 3.5a). A significant interaction between age and immigrant status remained in the model of likelihood of failing to breed in the reduced dataset (figure 3.5b, Est.: 0.2095±0.0740, $F_{1,1636.9}$=7.86, p=0.01, table 3.5b), but it is clear from figure 3.5b that immigrant stags were as likely to breed as natals at all ages, and more likely to breed from the age of 6.

Therefore in general, once paternity assignment bias is considered, immigrant males did have greater breeding success than natals. There was also a significant interaction between a male's age and his immigrant status on rut duration (table 3.4c, figure 3.6). Immigrant males in general rutted for shorter periods than natals; but this difference was less apparent in males aged eleven and older

Figures 3.4a and 3.4b: changes in breeding success with age, split by males' immigration status, for a) annual breeding success b) likelihood of failing to breed.



Figures 3.5a and 3.5b: Changes in breeding success with age, split by males' immigration status for sampled males only: a) annual breeding success, b) likelihood of failing to breed.

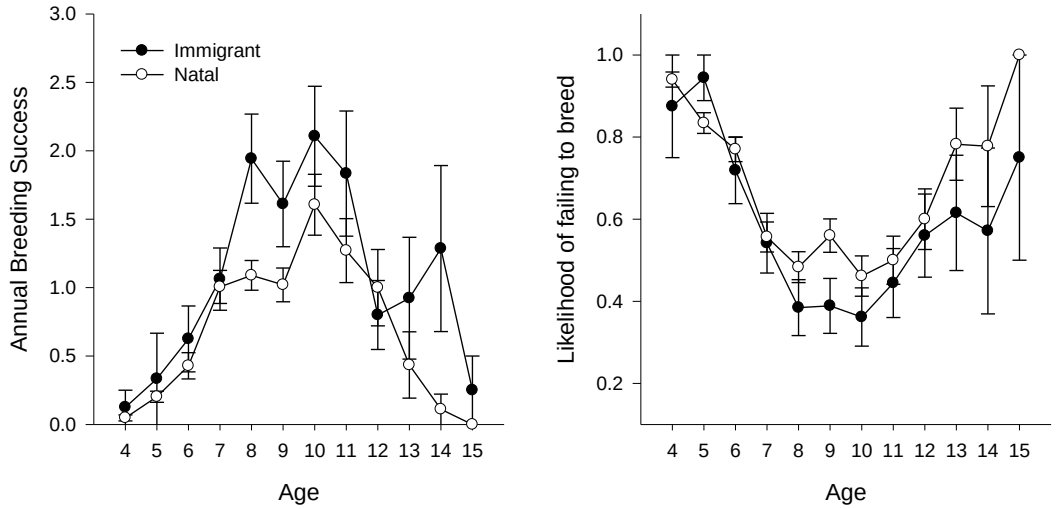


Figure 3.6: changes in rut duration with male age, split by whether males are immigrant or natal.

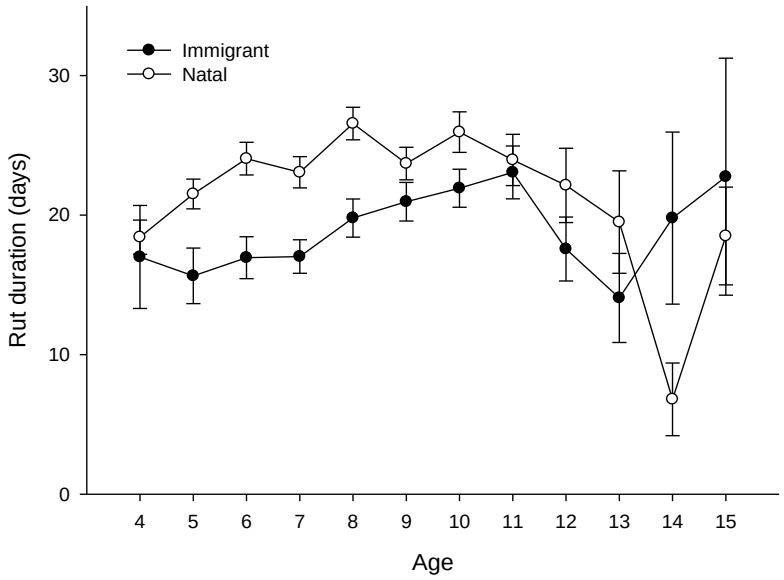


Table 3.4: results of the model of the individual-level models of male breeding success: a) annual breeding success, b) likelihood of failing to breed, c) duration of rut (days). Only the results of the minimum adequate models are given, but with marginally non-significant interaction term of immigrant male number by variance in oestrus date retained in the likelihood of failing to breed model. Estimates and standard errors are given, as well as F values, degrees of freedom and p values, for a Wald test, dropping individual terms from the full model. F tests for main effects of terms which occur in significant interactions are therefore not reported as these cannot be individually dropped from the model when the interaction is retained.

Variable	Estimate	S.E.	F value	d.f.	p value
a) Annual Breeding Success					
Intercept	-0.9709	0.0741			
Age	1.6190	0.1027			
Age^2	-0.0820	0.0052	244.26	1,2009.3	<0.01
Variance in oestrus date	0.0012	0.0006	4.25	1,1918.0	0.04
Immigrant status (natal)	0.0164	0.0928			
IM	-0.0119	0.0037			
Age*Immigrant status (natal)	-0.0918	0.0257	12.78	1,1988.5	<0.01
Age*IM	-0.0078	0.0022	12.03	1,2005.4	<0.01

Variable	Estimate	S.E.	F value	d.f.	p value
b) Likelihood of failing to breed					
Intercept	1.3530	0.1509			
Age	-2.836	0.2010			
Age^2	0.1420	0.0105	182.47	1,1916.0	<0.01
Variance in oestrus date	-0.0025	0.0018			
Immigrant status (natal)	-0.3519	0.0191			
IM	0.0126	0.0118			
Age*Immigrant status (natal)	0.3007	0.0661	20.71	1,1974.9	<0.01
Age*IM	0.0142	0.0047	9.08	1,1946.4	<0.01
IM*VO	-0.0006	0.0003	3.17	1,1799.4	0.08
c) Rut duration					
Intercept	2.7860	0.0396			
Age	0.3058	0.0417			
Age^2	-0.0165	0.0023	53.76	1,1919.1	<0.01
Immigrant (natal)	0.2582	0.0499			
IM	-0.0359	0.0155			
Age*Immigrant status (natal)	-0.0359	0.0155	5.37	1,2057.9	0.02
IM*Immigrant status (natal)	0.0156	0.0057	7.38	1,2057.1	0.01

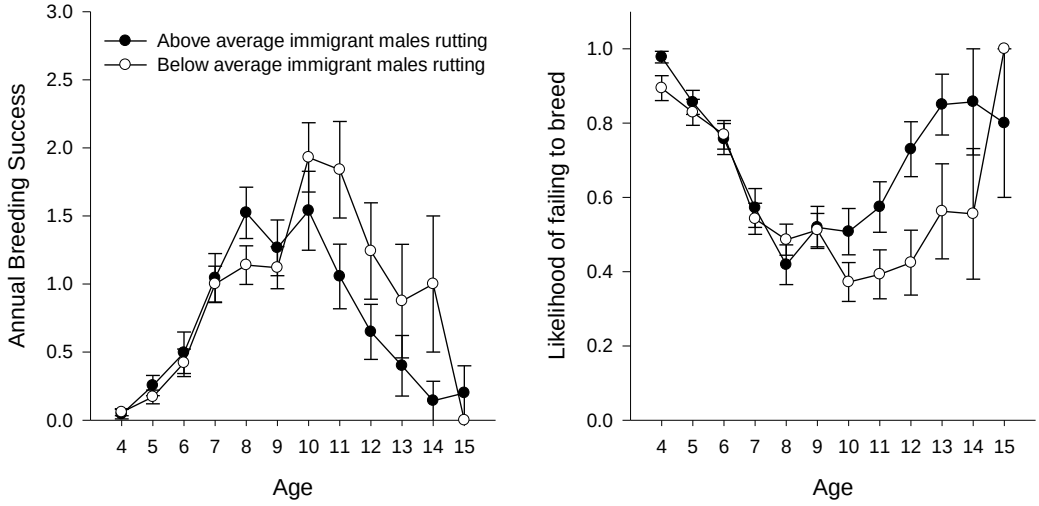
Table 3.5: minimum adequate models of individual based breeding success models, for sampled males only. Estimates and standard errors are given, as well as F values, degrees of freedom and p values for a Wald test, sequentially adding terms to fixed model.

Variable	Estimate	S.E.	F value	d.f.	p value
Annual Breeding Success					
Age	1.7030	0.1116	56.21	1,1489.3	<0.01
Age^2	-0.0915	0.0063	204.15	1,1488.3	<0.01
Immigrant (native)	-0.6122	0.1589	13.40	1,247.6	<0.01
No. immigrant males (IS)	-0.0116	0.0066	2.23	1,1613.6	0.14
Variance in oestrus date (VO)	0.0023	0.0010	5.34	1,1529.5	<0.01
Age*IS	-0.0106	0.0030	12.34	1,1534.3	<0.01

Variable	Estimate	S.E.	F value	d.f.	p value
Failure to Breed					
Age	-2.6460	0.2137	53.09	1,1568.5	<0.01
Age^2	0.1369	0.0110	148.04	1,1577.0	<0.01
Immigrant (native)	0.8533	0.2412	12.54	1,313.0	<0.01
No. immigrant males (IS)	0.0086	0.0127	3.37	1,1635.0	0.07
Variance in oestrus date (VO)	-0.0037	0.0020	1.41	1,1610.8	0.24
Age*Immigrant (native)	0.2095	0.0747	6.26	1,1635.9	0.01
Age*IS	0.0134	0.0050	7.53	1,1615.2	0.01
IS*VO	-0.0009	0.0004	6.01	1,1517.5	0.01

An increased number of immigrant males was associated with depressed average reproductive performance amongst males. We found a significant interaction between male age and the number of immigrant males rutting on both ABS (figure 3.7a, table 3.4a) and whether a male failed to breed (figure 3.7b, table 3.4b). Higher numbers of immigrant males rutting were associated with lower individual ABS, and higher likelihood of individual males failing to breed, suggesting greater competition for access to females. However, these effects were restricted to individuals aged 10-12. Given males in this population achieve maximum annual breeding success between the ages of 8 and 11 (Nussey *et al.* 2009), our results indicate increased competition affected predominately prime-aged males. Increased numbers of immigrant males were also associated with shorter rut durations of immigrant males: we found a significant interaction between whether a male was immigrant or natal, and the number of immigrant males holding harems on the duration of a male's rut (see table 3.4c, figure 3.8). Immigrant males rutted for shorter periods as the number of immigrant males holding harems increased, whilst natal males rutted for slightly longer, suggesting a greater effect of competition on the rut duration of more competitive males (on separating the analysis into immigrant and natal males, immigrant males: effect of number of immigrant males= -0.1667±0.0886, $F_{1,652.9}$=3.54, p=0.061, natal males: effect of number of immigrant males= 0.1418±0.0768, $F_{1.13971.}$=3.41, p=0.065).

Figures 3.7a and 3.7b: effect of the number of immigrant males on a) ABS and b) likelihood of failing to breed.



Finally, we found a trend suggesting the effect of increased numbers of immigrant males rutting was dependent on variability in the availability of receptive females (the interaction between the number of immigrant males rutting and variance in female oestrus date on the likelihood of an individual male failing to breed was marginally non significant: $F_{1,1799.4}$=3.17, p=0.08, see table 3.4b, figure 3.9a and 3.9b). When there were many immigrant males rutting, the likelihood of a male failing to breed decreased strongly as the variance in oestrus dates increased; in contrast when there were few immigrant males rutting, the likelihood of a male failing to breed increased slightly with variance in female oestrus. There was also a small, positive effect of variance in oestrus date on ABS (table 3.4a), but the interaction between the number of immigrant males rutting and variance in female oestrus had no significant effect on ABS.

Figure 3.8: the relationship between the number of immigrant males holding harems in each year and the length of time individual males spent rutting for i) immigrant males and ii) natal males.

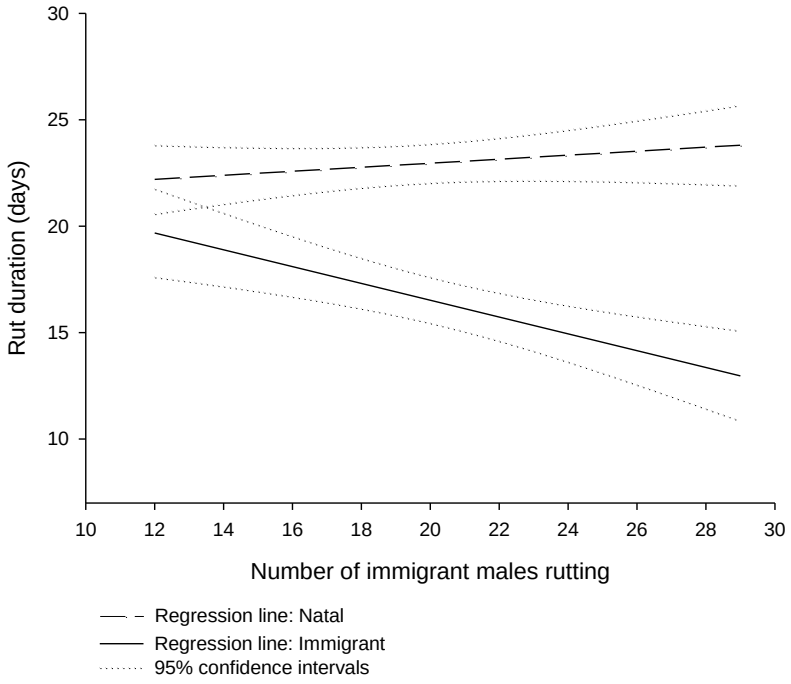
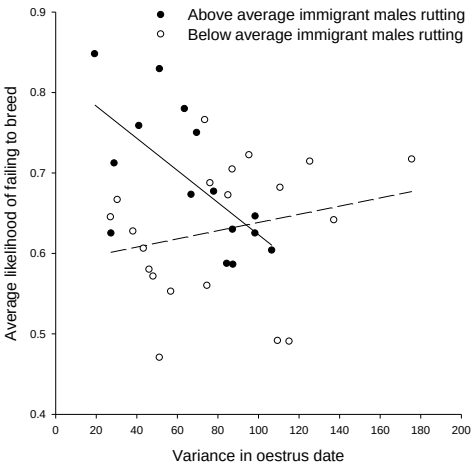
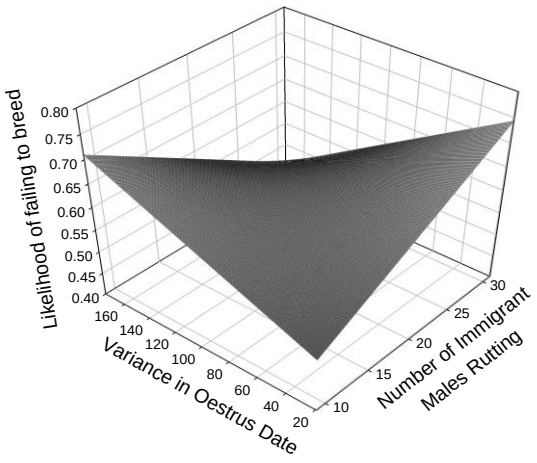


Figure 3.9a: effect of variance in oestrus on mean likelihood of failing to breed when the number of immigrant males is lower than average (open circles, dashed line), or equal to or above average (filled circles, solid line).

Figure 3.9b: model predictions of the interaction between variance in oestrus date and the number of immigrant males rutting on the likelihood of males failing to breed.





With the exception of the differences in fitness between immigrant and natal males discussed above, the individual level results were broadly consistent when using data from genotyped males only (table 3.5). Further, they were also consistent when using data from 1982 onwards only (table 3.6). We found three differences between the full study period and post-1982 datasets. Firstly, there was no main effect of variance in oestrus on ABS in the post-1982 dataset. Secondly in our model of rut duration, the interaction between a male's age and his immigrant status was no longer significant. In contrast we found a significant interaction between a male's age and the number of immigrant stags rutting on rut duration: however, when we examined the data, both these interactions differences are only apparent in the 14 and 15 age classes. These are very old males, records of which account for only 0.01% of data in the full dataset. Thirdly, there was no significant interaction between the number of immigrant males rutting and male age in the individual failure to breed model after 1982; however, higher numbers of immigrant males rutting still increased the likelihood of failing to breed. The general consistency between the full and post 1982 datasets suggests that changes in population demography after the release from culling have not greatly changed the effects of competition and temporal female availability. It should also be noted that although the percentage of calves assigned a father increased over time, the consistency of results before and after 1982 shows this appears to not have an important impact on our findings.

Table 3.6: minimum adequate models of individual based breeding success models, for 1982 onwards. Estimates and standard errors are given, as well as F values, degrees of freedom and p values for a Wald test, sequentially adding terms to fixed model.

Variable	Estimate	S.E.	F value	d.f.	p value
Annual Breeding Success					
Age	2.1470	0.1381	51.6	1,1438.2	<0.01
Age^2	-0.1090	0.0071	217.25	1,1428.4	<0.01
Immigrant (natal)	-0.0020	0.1519	0.10	1,363.4	0.75
No. immigrant males (IS)	-0.0156	0.0073	4.71	1,1613.1	0.03
Age*Immigrant (natal)	-0.1631	0.0440	12.84	1,5979.7	<0.01
Age^2*IS	-0.0004	0.0002	3.83	1,1475.1	0.05
Failure to Breed					
Age	-2.9870	0.2238	43.04	1,1522.9	<0.01
Age^2	0.1527	0.0118	153.61	1,1525.3	<0.01
Immigrant (natal)	-0.4533	0.2108	4.18	1,418.5	0.04
No. immigrant males (IS)	0.0106	0.0138	2.22	1,1657.7	0.14
Variance in oestrus date (V0)	-0.0029	0.0020	0.90	1,1370.2	0.34
Age*Immigrant (natal)	0.2857	0.0782	13.65	1,1657.1	<0.01
IS*VO	-0.0007	0.0004	3.09	1,1487.0	0.08
Rut duration					
Age	0.3110	0.0410	0.17	1,1499.3	0.68
Age^2	-0.0191	0.0025	47.24	1,1455.1	<0.01
Immigrant (natal)	0.3169	0.0563	33.13	1,482.4	<0.01
No. immigrant males	-0.0238	0.0077	0.02	1,1688.5	0.88
Age*IS	-0.0152	0.0079	0.03	1,1618.3	0.86*
Age^2*IS	0.0010	0.0005	5.90	1,1582.9	0.02
Immigrant*IS	0.0284	0.0087	10.73	1,1648.9	<0.01

*****near significant when dropping terms from full fixed model, $F_{1.1618.3}$=3.71, p=0.054; retained because Age^2*IS is significant.

Selection differentials

There was little evidence for a correlation between I_s and annual selection differentials on rut start date, rut end date or antler mass (rut start: -0.3112±0.2352, $F_{1,32}$=1.75, p=0.195, R=0.23; rut end: 0.2148±0.1938, $F_{1,32}$=1.23, p=0.276, R=0.19; antler mass: -0.1132±0.0864, $F_{1,32}$=1.72, p=0.199, R=0.23, see figure 3.10).

The interaction between the number of immigrant males rutting and variance in female oestrus was not significantly associated with annual selection differentials on either rut end date or antler mass (rut end: $F_{1,30}$=0.07, p=0.79, antler mass: $F_{1,30}$=0.18, p=0.67). However, there was weak evidence that the same ecological parameters which predicted I_s were also associated with selection on male rut start dates: we found a non-significant trend towards the interaction between the number of immigrant males rutting and variance in female oestrus in predicting annual selection differentials on rut start date ($F_{1,30}$=2.60, p=0.12, see figure 3.11). All selection differentials on rut start date were negative, indicating males in this population who rut earlier have higher reproductive success. This considered, we found selection became stronger as females were more asynchronous when few immigrant males were rutting, but weaker when females were more asynchronous when many immigrant males were rutting (see figure 3.11). The direction of this effect is consistent with our findings on how these parameters affected I_s.

Figure 3.10: the relationship between I_s and annual selection differentials on rut start date, rut end date and antler mass.

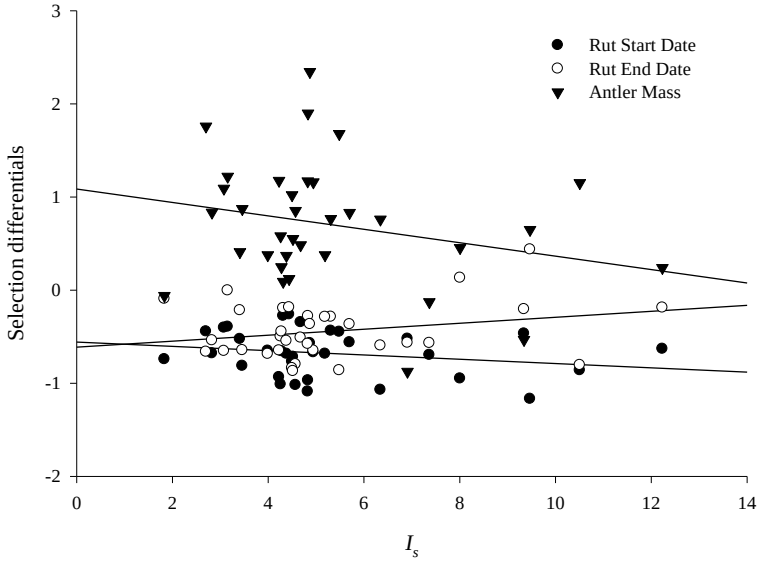
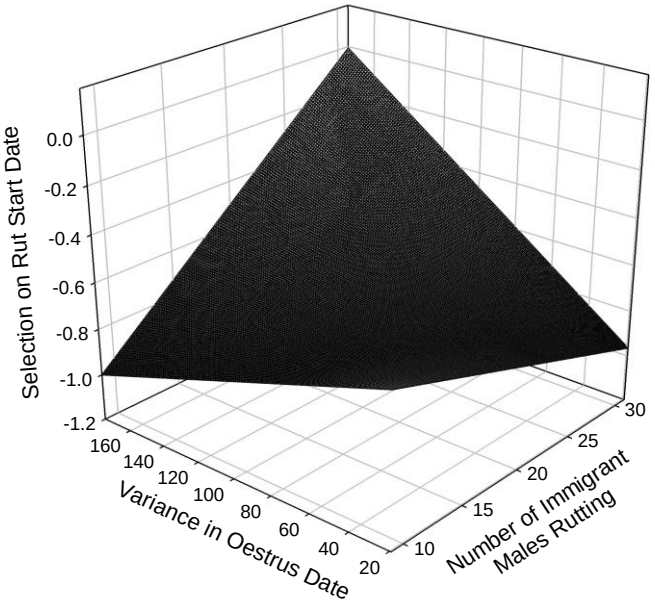


Figure 3.11: model predictions of the interaction between variance in oestrus date and the number of immigrant males rutting on annual selection differentials on rut start date.



3.4 Discussion

We have found that in this wild population of red deer, higher numbers of immigrant males rutting were associated with depressed individual annual breeding success and higher variance in male reproductive success, indicating stronger competition between males for access to females. The effect of this competition, at both the population and individual level, was dependent on the temporal availability of oestrous females. The same interaction between numbers of immigrant males and the temporal availability of females also showed some limited association with selection on one male trait - the date on which a male started rutting - but not selection on rut end dates or antler mass.

These results are likely to stem from changes in the ability of males to monopolize females when the spread of female oestrus dates is smaller or greater, and how these changes are dependent on the number of competitors which males face. Under low competition, increasing variance in female oestrus was, as predicted, associated with higher I_s and increased likelihood of males failing to breed: this can be explained if increasing variability in female oestrus dates enables the most dominant harem holding

males to monopolize and defend oestrous females more successfully, because they have sufficient 'handling time' to fertilize a female without loss of opportunity of access to other oestrous females (Ims 1988). However, contrary to our predictions, the effect of variance in female oestrus dates switched under high competition, to a negative association with I_s. When competition is greater, defence polygyny is predicted to break down into a form of scramble competition, as the energetic demands of maintaining harems or territories increases relative to the advantages (Geist 1982, Knell 2009); such a collapse has been empirically demonstrated in a number of species, including seed bugs (McLain 1992) and bitterling (Reichard *et al.* 2004b). Indeed, in this study, the rut durations of immigrant males were lower under high competition, perhaps suggesting more competitive males are becoming exhausted more quickly. In this scenario, as female reproductive availibility becomes more spread out over the rut, theory predicts the rate of appearance of new females becomes such that the cost of continued harem defence exceeds the benefits of obtaining an additional female (Emlen and Oring 1977). We suggest therefore that shorter rut durations of competitive males, and more instability in harems from competition, when coupled with increased variability in oestrus, widens the group of males that gain access to oestrous females during the rut, resulting in the reduced likelihood of males failing to breed and the reduction in I_s which we have observed.

Although effects of female synchrony on male reproductive success have been found in other studies (Say *et al.* 2001, Mendoza-Cuenca & Macías-Ordóñez 2009, Mysterud *et al.* 2008), to our knowledge, this is the first time that the interaction between variance in female oestrus date and male competition on variance in male reproductive success has been shown in a wild mammal. This is strong empirical evidence for the theoretical models of Ims (1988), which predict that in mammalian systems characterized by male competition and active searching for females, when female reproduction is asynchronous, variance in mating success will decrease with an increasingly male-biased sex ratio.

Competitive differences between males

The number of immigrant males rutting was more strongly correlated with I_s than the total number of males rutting, indicating that immigrant males have greater impact on the strength of competition. We have shown that, after addressing biases in paternity assignment, immigrant males have higher annual breeding success and are more likely to breed in any year; we therefore argue that immigrant males are stronger competitors than

natals. Clutton-Brock *et al.* (1997) suggested that males immigrating into the study population are stronger competitors than natal males because they experience lower population density in their early life, due to the continued culling regime outside the study area.

The effect of competition on male breeding success was dependent on the relative competitive abilities of those males rutting: as such it is clear that in understanding mating competition, it is not just the number of competitors, but also their relative competitive abilities that is important. Age or class-dependent sensitivities to demographic effects have been predicted where individuals have different competitive abilities and studies have suggested this may have important downstream effects on population dynamics (Mysterud *et al.* 2003, Dreiss *et al.* 2010, Pfister 1998). In this study, competition appeared to mainly affect the classes of males most likely to obtain breeding success (prime aged and immigrant males). This suggests that competition from immigrant males affects the ability of males to maintain harems, rather than other mating strategies, given that non-prime aged males that are unable to defend harems frequently use alternative tactics such as sneaking (Clutton-Brock *et al.* 1982).

Selection differentials

I_s showed little correlation with annual selection differentials on rut start dates, rut end dates or antler mass. Although I_s is defined as the *upper limit* for phenotypic change caused by variation in mating success, it is frequently used as a proxy for the strength of sexual selection (Klug *et al.* 2010a, as in Weatherhead 2008, Reichard *et al.* 2008, Twiss *et al.* 2007). A few authors have found correlates between I_s and the strength of sexual selection (Jones *et al.* 2004, McLain *et al.* 1992, and see review in Klug *et al.* 2010a), but many other studies have found non-significant relationships between I_s and selection differentials (see review in Klug *et al.* 2010a) and even some negative relationships: for example, Kelly (2008) reported a negative relationship between I_s and the strength of a selection gradient on a sexual trait in the insect *Hemideina crassidens*.

Klug *et al.* (2010a) have suggested that only under extremely restrictive conditions will I_s accurately predict the strength of sexual selection on male traits, and so vary across OSRs in the same way as actual selection on male traits. In this study, evidence for a positive correlation between I_s and selection differentials was limited, although the same ecological

factors affecting I_s were weakly correlated with selection on rut start dates. Rut start dates in males are strongly correlated with antler cleaning dates (the end of the growth period for antlers) and so are likely to be influenced by condition (Clements *et al.* 2010, Moyes *et al.* 2010, Lincoln 1992, Clutton-Brock *et al.* 1982). We have found that under high competition (many immigrant males rutting), selection is stronger on rut start dates when females are more synchronous. We suggest that if availability of receptive females is clumped in the early, peak, part of the rut, selection will strongly favour males who can hold harems in this period and so gain access to females. However, if female oestrus dates have a tail later into the rut, selection against rutting late will be relaxed, particularly as under high competition, competitive males appear to rut for shorter periods. In contrast, under low competition, dominant males are likely to be able to maintain harems throughout the period in which receptive females are available, and so selection always favours males who are able to begin rutting early enough to maximise their access to the peak of reproductive opportunities.

Our findings therefore show that using I_s to examine variation in the strength of sexual selection across changing environmental conditions has the potential to highlight the ecological factors relevant to actual selection. However, in general our study finds weak support for the utility of the metric I_s as a proxy for the strength of selection on sexual traits. The lack of positive correlation between I_s and selection differentials on antler mass is particularly surprising given antler mass is a secondary sexual trait known to be under positive directional selection (Kruuk *et al.* 2002b). It should be noted that potential problems exist with calculating yearly selection gradients on the traits used: for example, there is evidence that rut end dates in this population are related to the cessation of female oestruses, and therefore may be a trait not under strong selection in males (Moyes *et al.* 2010, Clements *et al.* 2010). Furthermore, data on antler masses are limited, and so within year estimates of selection may not be accurate. Such methodological problems are indeed why I_s is such an attractive way to measure changes in the potential for selection: identifying the relevant trait of interest when attempting to quantify sexual selection is challenging. In red deer, other sexually selected traits exist such as roaring or fighting ability which were not included in our selection analysis; ideally measuring sexual selection in totality would require measuring selection on all sexual traits. I_s clearly provides useful information on the reproductive skew amongst males, and as such the factors that affect the strength of competition between males. However, the assumption

that I_s is interchangeable with the strength of sexual selection, as made by numerous studies, should clearly be approached with caution (Klug *et al.* 2010a).

Comparison with previous findings

In the same population, but using behavioural paternity assignment, Clutton-Brock *et al.* (1997) found that a female-biased population sex ratio (measured in spring) was associated with increased skew in male reproductive success due to an increase in the number of relatively unsuccessful males, suggesting competition intensity declined when more females were available. We found some evidence of this: in a univariate analysis, I_s was lower when the rut sex ratio was more female biased. However, in the full model, this result did not remain independently of the number of immigrant males rutting, indicating that the number of immigrant males rutting accounted for the rut sex ratio effect we found. This is not surprising, given rut sex ratio is negatively correlated with the number of immigrant males rutting (see table 3.1). However, our analysis does therefore suggest that male density, particularly the density of immigrant males, has a greater effect on intensity of competition than the ratio of males to females. This may be because the female population has not yet reached sufficient numbers that harem size is constrained by males' ability to maintain harems and defend oestrous females, particularly when female oestrus dates are asynchronous. Further, if sex ratio *outsid*e the rut affects the number of immigrant males that enter the area to rut, the effect of sex ratio outside the rut on intensity of competition for mates found by Clutton-Brock *et al.* (1997) could be partially explained by changes in competition caused by the number of immigrant males rutting. In support of this, Clutton-Brock *et al.* (1997) found the number of males temporarily immigrating into the population to rut increased with an increasingly female biased sex ratio.

It should be noted that like many wild populations, the pedigree information from which absolute and variance in reproductive success is calculated in this study is not complete. There are two particular potential non-random sources of paternity loss: the bias in lack of sampling of immigrant males, particularly those which are young and therefore unlikely to have behavioural information; and secondly the change in our ability to assign paternity over time. Given that the denominator of I_s is the square of mean male reproductive success, any bias in paternity assignment which systematically affects the error around our estimate of this mean potentially biases I_s. For example, in years with a higher proportion

of immigrant males amongst the rutting male population, our ability to assign paternities is likely to be poorer because a greater proportion of candidate males will be genetically unsampled. As a result, the estimate of mean breeding success, and so I_s, is likely to be estimated with greater error. We have attempted to address the problem of incomplete pedigree informationby repeating analyses with subsets of data which illuminate this problem (post 1982 data and data on sampled males only). However, in general we have to expect missing genetic information to result in underestimation of variance in male reproductive success (Pemberton *et al.* 1992); we therefore may underestimate the effects of ecological parameters on the male breeding success and also potentially selection on male traits.

Climate

We found no climatic effects on I_s. Many studies have found changes in breeding phenology with climate that have the potential to indirectly impact sexual selection (*e.g.* Reale *et al*. 2003, Post and Forchhammer 2008, Isaac 2009), yet few studies have reported direct effects of climate on variance in male reproductive success and so sexual selection in wild populations. However, it is not clear whether this is because this has not been addressed or because negative results have not been published. We looked for evidence of immediate effects of climate during the rut on I_s, which could occur if climate affected mating behaviour (as in Twiss *et al.* 2007), with the expectation that rainfall or cold weather could affect deer movement and so the spatial distribution of females (see Chapter 2). However, we found none, suggesting such effects, if they exist, are relatively minor compared to the effects of demographic parameters on I_s in this population. It should be noted however that the proximate mechanisms of such effects are likely to be species specific, and more fine-scale measures of climate than used here may be necessary to detect changes in mating behaviour. For example, storm events, or low cloud may cause females to be more spatially clustered on particular days during the rut: more detailed information on such localised weather effects than available to our study may be necessary to detect such immediate behavioural patterns. Climate prior to the rut may also be important, if it causes changes in condition that affect the patterns of mate competition or availability of oestrous females (Isaac 2009, Weatherhead *et al.* 2009, Cockburn *et al.* 2008). Further work is clearly needed in wild populations to investigate how climate affects the distribution of male reproductive success and whether this impacts on sexual selection.

Conclusions

Here we have demonstrated how relatively small inter-annual fluctuations in ecological factors, outside of periods of significant demographic change, can have significant effects on variance in male mating success in the wild. Our findings have important implications for our understanding of the evolution of sexually selected traits in wild populations and demonstrate a potentially important mechanism in the maintenance of genetic variation in such traits. Ecological theory tells us that operational sex ratio will be the primary determinant of the strength of sexual selection (Emlen and Oring 1977), yet we have found that in a heavily female-biased population, the absolute number of males is more important, and that differences in the competitive ability of those males are key. Critically, we have also shown that effects of competition depend on the monopolizability of females; it is clear therefore that future studies of ecological effects on sexual selection need to consider multiple factors and their interactions. Finally, our study demonstrates empirically the limitations of using standardized variance in mating success to capture the strength of sexual selection. The measurement of sexual selection is a challenging issue in the sexual selection literature, and empirical studies such as this, using not just metrics of selection but attempting to measure actual selection, are likely to be important in its resolution.

Chapter 4:

The variable stag: individual differences in the vocal reaction norms of wild red deer

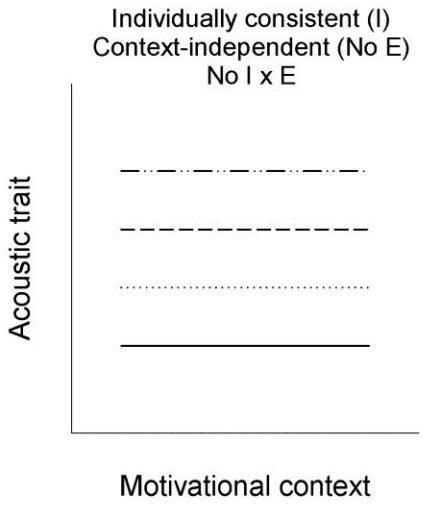
4.1 Summary

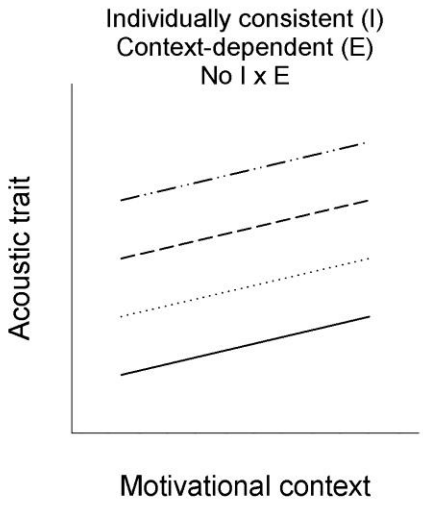
The use of behavioural reaction norms (BRNs) has recently been advocated to integrate studies of personality and plasticity. BRNs allow simultaneous assessment of between-individual variation in trait mean, population-level average plasticity and variation in plasticity. Further, they allow the strength of selection on each of these to be estimated, giving important insights into the evolution and maintenance of variation between individuals. In contrast to life-history traits, few studies have demonstrated individual variation in plasticity in BRNs, or the fitness consequences of such variation, particularly for sexually selected acoustic signals. We examine variation in minimum formant frequencies, an acoustic cue to body size, in roars produced by wild red deer, and how they vary with social environment: specifically the presence or absence of an oestrous female, the size of a male's harem and the length of time the male has spent rutting. We show significant levels of intra-individual variation in acoustic behavioural reaction norms in not just males' mean values for the traits, but also males' responses to changes in context. Finally, we test for selection acting upon this variation.

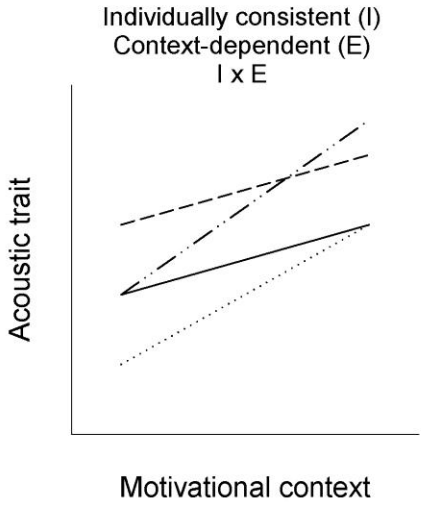
4.1 Introduction

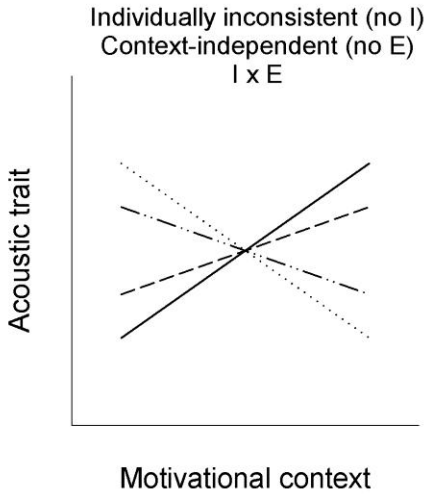
An important unresolved question in both evolutionary biology and behavioural ecology is the maintenance of variation within and between individuals, and whether this variation is adaptive (Endler 1986, Réale *et al.* 2010). Consistent individual differences in phenotype are commonly observed in animal systems, as are differences in phenotypic plasticity, a change in phenotype in response to a change in context or environment (Cam *et al.* 2004, Sih *et al.* 2004, Pigliucci 2005, Réale *et al.* 2007). Context or environment in this sense can refer to a wide number of changes: social situation, habitat, internal state or even in time, such as over a mating season. Phenotypic plasticity, and variation in phenotypic plasticity, is often described using reaction norms, functions relating a phenotypic trait to an environment or contextual gradient; this reaction norm approach has provided important insights into the maintenance of genetic variation within populations (Via *et al.* 1995; Pigliucci 2005). The application of an individual-level reaction norm approach in evolutionary and behavioural studies allows simultaneous consideration of between-individual variation in average phenotype, population level average plasticity and individual variation in plasticity (see figure 4.1, Nussey *et al*. 2007, Dingemanse *et al.* 2009). To date, in wild vertebrates, reaction norms have most commonly been applied to life history traits or morphological traits, revealing that individual variation in plasticity (or individual by environment interactions, 'I x E') is apparently common and reaction norms may be under natural selection (Nussey *et al.* 2005c, Brommer *et al*. 2005, Martin *et al.* 2011).

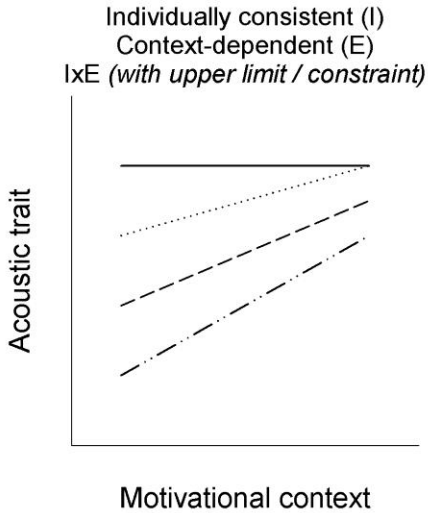
Figure 4.1: Schematics showing reaction norms for four individuals expressing a behavioural trait (in this case acoustic) measured several times across a change in environment (in this case, motivational context). If consistent inter-individual variation exists in a trait, but individuals do not respond to context, behavioural reaction norms will appear horizontal, as in 4.1a. If individuals vary in elevation and all respond in the same way to context, then rank of individuals will remain the same over the range of the context, as in 1b. In both these situations therefore consistent differences exist between individuals in average behaviour, but not response to context, *i.e.* I but not I x E. However, individuals may vary in their responses to context, as in 4.1c, and 4.1d, so that there is an interaction between individual and context, *i.e.* IxE. Finally, individuals may vary in response to context, but with some constraint, so that for example, in 4.1e, individuals already producing high values of the trait cannot increase that trait as the context changes.

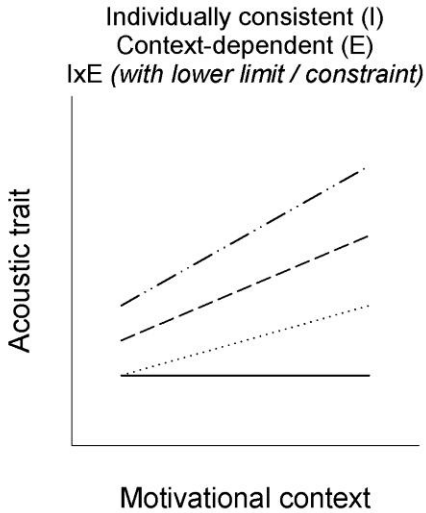

Individually consistent (I)
Context-independent (No E)
No I x E
Acoustic trait
Motivational context


Individually consistent (I)
Context-dependent (E)
No I x E
Acoustic trait
Motivational context


Individually consistent (I)
Context-dependent (E)
I x E
Acoustic trait
Motivational context


Individually inconsistent (no I)
Context-independent (no E)
I x E
Acoustic trait
Motivational context


Individually consistent (I)
Context-dependent (E)
IxE (with upper limit / constraint)
Acoustic trait
Motivational context


Individually consistent (I)
Context-dependent (E)
IxE (with lower limit / constraint)
Acoustic trait
Motivational context

There is also however rapidly growing interest in applying a reaction norm approach to understand variation in behavioural traits and their responses to context ("Behavioural Reaction Norms" or BRNs, Sih *et al.* 2004, Smiseth *et al.* 2008, Dingemanse *et al.* 2010), *i.e.* in traits which respond quickly to changes in the social environment and are reversible over time (Smiseth *et al.* 2008). BRNs allow estimation of whether, and how, selection acts upon components of behavioural responses, and are therefore at the interface between behavioural ecology and quantitative genetics, providing a basis on which to study the heritable component of response functions (Smiseth *et al.* 2008). The critical consideration is that under a behavioural reaction norm framework, both inter-individual and intra-individual variation are considered meaningful, rather than just noise. Therefore, we can consider how selection acts upon both elevation and slope of the reaction norm, and in particular whether they can evolve independently (Dingemanse *et al.* 2009).

Theoretical models suggest that inter-individual variation in behavioural responses could be adaptive or may result from constraints, *i.e.* limits to plasticity (Dingemanse *et al.* 2010). However, evidence for individual variation in behavioural plasticity remains limited and few studies have been able to relate individual differences in behavioural reaction norms to reproductive fitness (Dingemanse 2009; Kontiainen *et al.* 2009). In this study, we measure inter- and intra-individual variation in formant frequencies of vocalizations of wild male red deer, *Cervus elaphus*. We show that individuals differ not only in average value for the trait, but also in plasticity of response to context. Finally, we test for selection on not just the trait but also plasticity in the response of the trait to changes in the social environment.

Male vocalizations are an important component of sexual selection in many species, signalling information about male phenotype relevant to both male-male competition and female choice (Andersson 1994a). Acoustic signals are expected, on average, to be honest in order for stable communication to evolve (Bradbury and Vehrencamp 1998, Botero *et al.* 2010); in general, sexual signals may be fixed by some anatomical component (*e.g.* by body size, such as in the acoustic signals of anurans, Gerhardt 1994) or constrained to a certain magnitude by costs of production or risks of escalation (for example, the tail of *Euplectes jacksoni*, Andersson 1994b). However, recent theoretical work has explored polymorphic sender codes: *i.e.* sender codes in which individuals in the same state produce signals of different intensity (Botero *et al.* 2010), and evidence for this has been found in the wild (*Hirundo rustica*, Muñoz *et al.* 2008). This variation can potentially

arise due to errors in self-assessment by the sender, attempts to deceive receivers through dishonest signalling, or, intriguingly, as a result of consistent individual differences between individuals, *i.e.* personality (Botero *et al.* 2010). Further, where signals are temporary, such as vocalizations, they may also exhibit a substantial plastic element, potentially varying with condition and motivation, resulting in high levels of not just inter- but also *intra*-individual variation (*e.g.* in primates, Elowson and Snowdon 1994, Mitani and Brandt 1994; *Capreolus capreolus*, Reby *et al.* 1999, Rukstalis *et al.* 2003; *Suricata suricatta,* Hollén and Manser 2007; *Dama dama,* Vannoni and McElligott 2009; *Canis lupus familiaris*, Taylor *et al.* 2009). Indeed, substantial variation in acoustic signals often exists which is poorly captured in regression models (Botero *et al.* 2010, *e.g.* Sanvito *et al.* 2008). Despite this apparent variation, relatively few studies have considered variation in sexual signals over short timescales, instead assuming stability of signals over the breeding season (Griffith and Sheldon, 2001, Vannoni and McElligott 2009). Of those studies which have considered such short-term variation, the majority have concentrated on variation in the rate of vocal signalling, and shown responses to social context (Berger and Cunningham 1991, McElligott and Hayden 1999) and fewer studies have addressed context-dependent variation in the acoustic structure of sexually selected vocalizations (although see Galeotti *et al*. 1997, Vannoni and McElligott 2009). To our knowledge, no study has examined the possibility that individuals might differ in their acoustic response to salient environmental cues or internal state.

Formant frequencies of vocalizations, the resonant frequencies of air in the vocal tract, have recently received much interest as the key acoustic parameter providing reliable information on body size in mammals, because of the close relationship between spacing of formant frequencies (formant dispersion) and the length of the vocal tract producing them (Fitch 1997, Riede and Fitch 1999, Fitch 2003, Harris *et al.* 2006, Sanvito *et al.* 2007, Vannoni and McElligott 2008, Charlton *et al.* 2009). In red deer, males retract their larynx when roaring to extend the vocal tract, but maximum extension of the vocal tract is constrained by the fixed position of the sternum, generating a minimum formant dispersion which is an honest signal of body size (note the extension of the neck in the roaring stag depicted in figure 4.2, and see Reby and McComb, 2003a). Maximum vocal tract length (VTL) in red deer is highly correlated with body weight (Reby and McComb, 2003a) and is an important sexual signal: males use formants frequencies to assess competitors, and playback experiments using modified roars have shown oestrous females prefer roars modified to indicate longer vocal tract lengths (Charlton *et al*. 2007a, Reby *et*

al. 2005). However, there is evidence that males may not always fully extend their vocal tract. Playback experiments have found males adjust their apparent VTL in response to the formant frequencies of their opponents (Reby and McComb 2003a, Reby *et al.* 2005) and Reby and McComb (2003b) have suggested that extension is greater immediately after herding oestrous females than at other times. This potential intra-individual variation in formant dispersion has received little attention in any species (although see Vannoni and McElligott 2009).

Figure 4.2b: A male red deer, 'TKN99', roaring. Note the extension of the neck. (Photo credit: Martyn Baker)

In this study we investigate individual variation in formant dispersion, and so apparent VTL, in wild red deer and how apparent VTL varies with context using BRNs. We relate repeated measures of apparent VTL across the rutting period to three measures of context: (1) whether or not the male is defending an oestrous female within his harem at the time of recording, (2) the size of his harem, and (3) how many days the male had been rutting when VTL was measured. We expect (1) and (2) to reflect the motivational context of the male's situation and (3) to reflect intrinsic state or condition. The rate of roaring by male red deer has previously been shown to be depend on whether the male was harem holding, the dispersion of females within a harem, and time within the rut (Clutton-Brock and Albon 1979), but to date the effects of context on apparent VTL have not been tested in the wild. We assumed linear reaction norms: comprising an elevation (average apparent

VTL) and a slope (behavioural plasticity) for each individual. We test whether individuals differ in their elevation and slope, and whether elevation and slope are correlated for each context variable in order to determine which of the differing scenarios illustrated in Figure 4.1 provide the best fit to our data. We then go on to examine selection on apparent VTL and behavioural plasticity in this trait by testing associations between a male's annual breeding success and apparent VTL at the average (elevation) and context-dependent (slope) levels.

4.2 Methods

Study population

Roars were recorded from males in a wild population of red deer on the Isle of Rum, Scotland. The population has been intensively studied since the early 1970s; all individuals are individually recognisable from artificial markings or natural idiosyncrasies. Red deer are polygynous, with a harem defence mating system, whereby males compete to defend groups of females and mate with those that are in oestrus. Fighting is costly; therefore males use visual and acoustic displays to assess potential competitors and avoid fighting with males they are unlikely to defeat (Clutton-Brock *et al.* 1979). During the breeding season, or 'rut', which occurs from September 15th- November 15th, daily censuses of the study area are conducted to record harem size and composition, noting the identity and location of all females and all males holding a harem (defined as defending at least one female). Females come into oestrus for approximately 24 hours, and most females only mate once (Guinness *et al.* 1971). The presence of oestrous females within harems can be determined from behavioural cues: chivvying by males (harassment), being in an unusual location, being mounted or served (*i.e.* the male was seen to ejaculate), or straining, as occurs after service (F. Guinness, *pers. comm.*, Clutton-Brock *et al.* 1982). During May/June, when calves are born, females are monitored closely to obtain information on birth date and weight of calves, as well as to take genetic samples for paternity analysis (see below). We therefore have accurate information on ages of all individuals born to the population. A number (29 in 2008) of immigrant males also enter the study area during the rutting period, and the ages of these immigrant males can be estimated from appearance. Analyses have shown no difference in the age-trajectory of male reproductive performance for immigrant and natal males, indicating our estimates of ages of immigrant males are likely to be accurate (see Chapter 3).

Figure 4.3 Andrew Fisher (field assistant) waiting to record a male, "CLT95". (Photo credit: Fiona Guinness)

Estimation of apparent vocal tract length (VTL)

Recordings were made using a Telinga Pro.5 stereo microphone mounted in a clear polycarbonate parabolic reflector measuring 53cm in diameter (see figure 4.3) The microphone was linked to a DA-P1 professional DAT recorded loaded with Sony PDP Pro DAT tapes. Roars were recorded opportunistically from 17 males rutting in 2008, aged 6-13 years (mean 10.12). The males recorded sired 71% of calves conceived in that year to which a father could be assigned; therefore they make up the majority of successfully rutting males in our study population. Six of the males recorded sired no offspring in the rut of 2008, therefore we have data on not just successful but also unsuccessful males. Males were recorded over different contexts and timepoints: nine males were recorded with and without an oestrous female (3-39 recordings per male per context); twelve males were recorded with more than one size of harem (1-26 recordings per male per harem size) and all but one male was recorded on more than day (1-19 recordings per male per day). The number of days between when a male was first recorded roaring until the last occasion on which he was recorded was on average 5.47 (range 1-10).

For each stag no less than 9 bouts of roars were recorded (mean 17.64), giving a dataset containing 298 bouts for analysis. A 'bout' is defined as a number of roars produced

without fully closing the mouth, consisting of 1-11 roars (Reby and McComb 2003a, Reby and McComb 2003b). Given previous studies have suggested that stags put most effort into the production of the first roar from each bout, that the plateau reflecting maximal vocal tract extension is not always reached in later roars, and the number of roars per bout is highly variable, we selected the first roar from each bout to standardize comparisons between males (*sensu* Reby and McComb 2003a). We also excluded "harsh roars", which are usually louder and have less formant modulation than "common roars" (Reby and McComb 2003a), and identified and discarded two 'lazy roars' from the dataset, in which formant frequencies do not fully plateau (see Reby and McComb 2003b).

Formant frequencies were extracted using the Praat package (Boersma & Weenink, 2010). As a male lowers its larynx to extend its vocal tract, the formant frequencies in a roar decrease to a minimum plateau. We extracted the first eight formant frequencies at 0.05s intervals throughout a roar and used these to identify the 0.5s interval containing this minimum plateau. Formant parameters were set as: time step: 0.05s; maximum number of formants: 8; maximum formant: 1900Hz; window length: 0.1s; pre-emphasis: 6000Hz (taken from Charlton *et al.* 2007a). Formant frequencies were then averaged over the plateau. The maximum VTL during the roar was then estimated from the minimum frequencies F_i as follows. First, the overall formant frequency spacing ΔF was derived by finding the best fit for the equation:

$$F_i = \frac{2i-1}{2}\Delta F$$

The apparent VTL can then be estimated from:

$$VTL = \frac{c}{2\Delta F}$$

Where c ($350ms^{-1}$) is the approximate speed of sound in a mammal vocal tract (Titze, 1994). Full details of the methods used to derive apparent VTLs are available in Reby and McComb (2003a).

Paternities

Calves are caught shortly after birth and tissue samples taken for genotyping. Of the males recorded in this study, seven were immigrant to the population and therefore not caught at birth, however, of these, four have been sampled from cast antlers. Individuals were genotyped at up to 15 highly variable microsatellites. Paternities were assigned using the

programs MasterBayes (Hadfield *et al.* 2006) and COLONY2 (Wang and Santure 2009) with greater than 80% individual confidence (see Walling *et al.* 2010 for full details). These programs also allow assignment of paternities to the remaining three ungenotyped males in this study, through use of phenotypic information on age, harem holding and antler size in MasterBayes, and assignment of half sib groups in COLONY2 (see Walling *et al.* 2010 for details): this allowed us to gain estimates of annual breeding success for all males recorded.

Statistical Analyses

Modelling variation in reaction norms for apparent VTL

We used both linear models (LMs) and linear mixed effects models (LMMs) to examine inter-individual variation in male apparent VTLs (elevation, I) and to determine the effects of three context variables - the presence of an oestrous female in the male's harem, the male's harem size and the number of days spent rutting - on apparent VTL (effect of environment, E). We tested for an overall association between changes in apparent VTL and each context variable, and also for inter-individual variation in the change in males' apparent VTL with each context (I x E, see Figure 1).

In order to test for between individual differences in behavioural plasticity, the use of LMMs including random regression terms have been advocated (Dingemanse *et al* 2009, Martin *et al*. 2011). In such models, fitting random effects for individual and an individual-by-context (or environment) interaction estimates the variance in, and correlations between, individual reaction norm elevations and slopes. A mixed model framework also has the advantage that it allows pseudo-replication associated with repeated measures of individuals or time points to be accounted for when testing fixed effects (Pinheiro and Bates 2000). However, although the significance of random effects can be evaluated using likelihood ratio tests, these have the potential to be unreliable for small sample sizes (Bolker *et al.* 2008). With relatively small behavioural data sets, it is very unlikely that tests for variation in behavioural responses to different context variations could be conducted simultaneously in the same model. However, this is important to establish whether responses vary independently of one another. We therefore measured variation in individual elevation in apparent VTL and slope of response to the three context variables using both the random regression LMM approach and a standard

LM approach, so that we could test slopes for different context variables in the same model. All analyses were conducted using Genstat v11.1 (VSN International, Hemel Hempsted, UK) or R 2.8.1 (R Development Core Team 2008).

Linear mixed models (LMMs)

In LMMs, day of recording (days since September 1st) was fitted as a random effect to account for changes in the competitive structure of males over the breeding season (see Clutton-Brock *et al.* 1982). We fitted the context variables as fixed effects: whether the male was defending an oestrous female on the day of recording (1/0); the male's harem size; and the day of recording in relation to when the male began rutting; as well as male identity. Male identity and day of measurement were fitted as cross-classified random effects. It should be noted that because only one record of the presence of oestrous females within a harem, and harem size, was available per day, we could only test for variation in slope within an individual, and not within a day nested within an individual, hence day was fitted as a second random effect, and not nested in individual. We tested for variation in individual responses by adding a random interaction term between male identity and the context variable. The correlation between intercept and slope was also modelled at this stage. Each context variable was considered in a separate LMM because the model would not converge when variation in responses to different contexts were fitted simultaneously in the same model. Significance of fixed effects was tested using Wald statistics and the significance of random effects by comparing models with likelihood ratio tests (LRTs). Significance of intercept-slope correlations were estimated by comparing the model using LRTs to one with no correlation term.

Linear models (LMs)

In the first linear model, male identity only was fitted as a factor, to test for differences between males in average apparent VTL, *i.e.* differences in elevation (I). In the second linear model we fitted presence of oestrous female, harem size and days since starting rutting as fixed effects. This was to test for overall changes in apparent VTL in response to context (E). In the final model we then fitted interactions between i) whether the male was defending an oestrous female and male identity; ii) the male's harem size and his identity and iii) the day of recording in relation to when the male began rutting and his identity; these were fitted as fixed effects to test for inter-individual variation in males'

responses to different context variables (IxE). All interactions were fitted in the same model. Models were constructed in this forward step-wise manner in order to allow assessment of significance of main effects of context variables independent of the interaction term. Significance of terms was assessed using Type II F tests, dropping each term from the full model. In these models, day of recording was not fitted, as significance of correlated parameters (*i.e.* days since the start of rutting) could not be assessed.

Selection analyses

We tested for correlations between annual breeding success and males' average apparent VTL (*i.e.* elevation), the coefficient of each male identity from the linear model of apparent VTL against male identity. Secondly, we tested for correlations between ABS and males' responses to context variables *i.e.* the slope of response for each male. This was taken from the coefficient of the interaction term between context variable and male identity for each male, from linear models of apparent VTL against the interaction between context variable and male identity. For this, each linear model used to generate these coefficients could only contain one context variable, because of differences in sample sizes between the variables. Further, although day of recording could not be fitted in the linear models used to test significance of context variables, given the LMMs revealed the significance of this term (see results), it was included as a main effect in linear models when extracting coefficients to account for the fact that males rutting earlier in the season tend to have higher annual breeding success (Moyes *et al.* 2010). We used generalised linear models with a quasi-Poisson error structure, with annual breeding success as the response variable. Age and its quadratic term were fitted as fixed effects in all models. We then fitted average apparent VTL, and the slope of a male's response in apparent VTL to each context variable. By fitting the elevations and slopes in response to each context variable in the same model, we are examining selection on each term independent of the others, *i.e.* selection on elevation independent of selection on slope, and selection on slope of response to context independent of selection on slope of response to other context variables.

4.3 Results

Variation in reaction norms for apparent VTL

Individual variance (I)

There were significant differences between males in average apparent VTL, *i.e.* elevation. Male apparent VTLs ranged from 68.8 to 75.4cm, with an average standard deviation of 2.60cm around this average, In the linear model framework there was a significant effect of male identity on apparent VTL ($F_{16,281}$=5.599, p<0.001). In the LMM, we again found significant inter-individual variation: in each model, males varied significantly in elevation (LRTs for all three models: X^2_1>27, p<0.001, table 4.1).

Effect of context variables (E)

There were no overall significant effects of having an oestrous female on apparent VTL ($F_{1,278}$=0.001, p=0.992) or harem size ($F_{1,278}$=0.462, p=0.497) in the linear models. There was a significant negative effect of days since the male first held a harem (in minimal model, *i.e.* with presence of an oestrous female and harem size removed, Estimate=-0.172±0.0618, $F_{1,278}$=7.705, p=0.006), indicating reduced extension of vocal tracts later in the rut. Including these terms added little to the variance in apparent VTL explained by male identity (R^2 model = 0.26, R^2 model with just male identity = 0.24). In the LMMs, there was again no significant fixed effect of the presence of an oestrous female (in model with identity and day only as random effects and all context variables as fixed effects, Estimate= 0.046±0.046, $F_{1,237.8}$=0.01, p=0.918), nor harem size (Estimate= 0.026±0.034, $F_{1, 289.2}$=0.59, p=0.443). In this model, there was also no significant effect of days since the onset of rutting (Estimate=-0.029±0.039, $F_{1,17.1}$=0.54, p=0.472). This difference with the linear model presumably results from temporal variance in apparent VTL being accounted for by the inclusion of days since September 1st as a random effect. Figure 4.4 shows graphically that there is no clear average response of apparent VTL to these context variables, but instead there is considerable variation in individual response, as detailed below.

Table 4.1. Variance components - the variance in apparent VTL explained by day of recording, male identity, and the interaction between male identity and the context variable - estimated using LMMs for each of the three context variables: a) presence of an oestrous female, b) harem size and c) the number of days a male had been rutting. Chi squared values and p values refer to the results of likelihood ratio tests performed on sequential models.

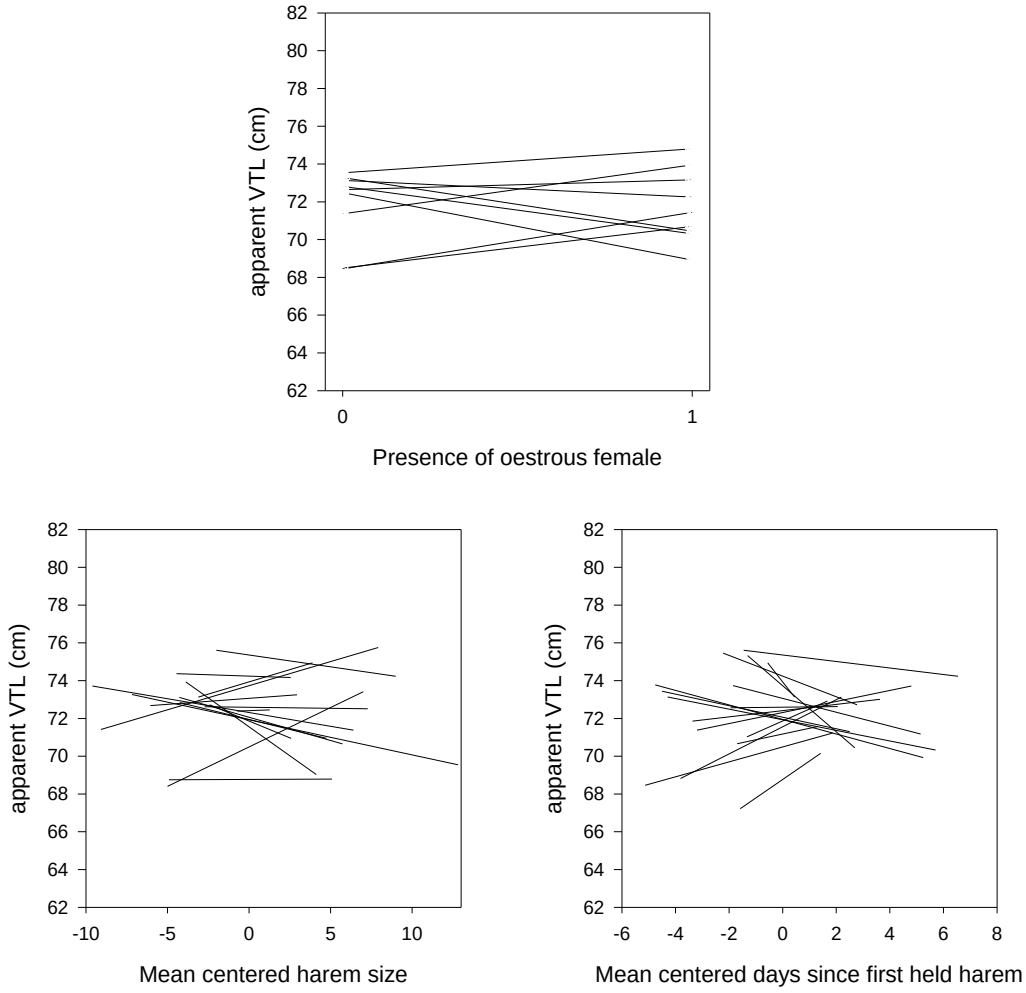
(a) Presence of oestrous female				
Random effects				
Term	Variance /Correlation	X^2	d.f.	P
Day	1.2171	21.71	1	<0.001
Individual	2.7881	27.71	1	<0.001
Individual x context	3.8614	10.00	1	0.007
Correlation	-0.789	5.05	1	0.025
Residual	5.9786			
(b) Harem size				
Random effects				
Term	Variance/Correlation	X^2	d.f.	P
Day	1.2842	21.45	1	<0.001
Individual	3.3617	28.35	1	<0.001
Individual x context	0.0595	31.43	1	<0.001
Correlation	-0.678	6.41	1	0.011
Residual	5.2880			
c) Time spent rutting				
Random effects				
Term	Variance/Correlation	X^2	d.f.	P
Day	0.7889	18.36	1	<0.001
Individual	3.7991	27.33	1	<0.001
Individual x context	0.0243	5.96	1	0.051
Correlation	-0.915	5.61	1	0.016
Residual	6.2398			

Individual variation in response to context variables (I x E)

There were significant differences between males in slope, *i.e.* in the response of apparent VTL to each context (linear models: significant interaction between male identity and presence of an oestrous female $F_{8,245}$=8.29, p<0.001, between male identity and harem size, $F_{14,285}$=5.416, p<0.001, and between male identity and days since a male first held a harem, $F_{11,285}$=4.960, p<0.001). This indicates there were significant inter-individual differences in response to context, which are apparent in figure 4.4. Given all three interactions between male identity and the within-individual rut covariates were significant when fitted in the same model, the three rut variables were independently related to inter-individual variation in apparent VTL. Including these interactions in the linear model explained a further 32% of the variation observed in male apparent vocal tract length compared to a model with only main effects of day, individual and within-individual rut covariates (R^2 full model (IxE) =0.58, R^2 model with no interactions between identity and rut covariates (I and E) =0.26, R^2 model with just male identity (I) =0.24). In the LMM framework, these findings were supported. Males varied significantly in the slope describing their change in VTL with the presence of an oestrous female and harem size (LRTs: X^2_1>10, p<0.008, see table 4.1a and 4.1b). For days since a male first rutted, the interaction between individual and context was very marginally non-significant (X^2_1=5.96, p=0.051, see 4.1c). We also re-estimated the context variables as fixed effects in the linear mixed models when the random slope term was fitted, to check for any bias in our findings arising from assessing fixed effects when random slopes were not included (Wolfgang and Forstmeier 2008). The results did not qualitatively differ (presence of oestrous female: -0.357±0.755, $F_{1,12.3}$=0.22, p=0.645, harem size: -0.005±0.034, $F_{1,202.4}$=0.02, p=0.877, days since started rutting: 0.015±0.043, $F_{1,11.6}$=0.12, p=0.734). Using the LMM, we were able to identify significant correlations between average VTL (intercept for male identity) and the response to each context variable (slope for each male, see table 4.1). For each context variable, this correlation was negative, indicating males with larger apparent VTLs on average were more likely to reduce VTL in response to the presence of an oestrous female, an increase in harem size or over the period of the rut; but that males with smaller apparent VTLs on average were more likely to increase VTL in response. This is clear from the crossing patterns of reaction norms in figure 4.4. Further, however, it suggests a constraint to apparent VTL may prevent males which on average signal with a larger apparent VTL from increasing VTL in response (*cf* figure 4.1e). In general, we have found significant I, and also significant IxE, potentially with

constraint; therefore our data is most in accordance with the scenario illustrated in either figure 4.1c or figure 4.1e.

Figure 4.4: a) variation in the change in apparent VTL in the presence of an oestrous female in different males - lines link means of individual males under the two contexts; **b)** variation in the effect of harem size (mean centered) on apparent VTL of males; and **c)** variation in the effect of the number of days since the male began rutting (mean centered) on apparent VTL. Lines shown in b) and c) are regression lines for the effect of harem size or days since the male began rutting on apparent VTL for each male.



A potential concern in this analysis is that the relatively small sample size and the unbalanced nature of the data may cause us to confuse stochastic variation with significant IxE. To check whether this was likely to be a problem in this analysis, we ran a simple simulation, using the context variable with the most restricted dataset (presence/absence

of oestrous female). In this simulation, each male in the observed dataset was assigned a random set of values of vocal tract length for each level of the context variable (presence/absence of oestrous female) such that the overall mean and standard deviation of the distributions of simulated values were the same as in the observed data for each level of the context variable. Males were assigned the same number of observations for each context level in the simulation as were recorded for that male in the observed dataset, in order to mimic the unbalanced data structure in the observed data, we then tested for the presence of IxE in the simulated dataset, in the same way as we had for the observed dataset. In the simulation, we found no evidence for IxE (χ^2=0.02, p=0.990 on two degrees of freedom) and further, that the correlation between elevation and slope in the simulation was zero. This suggests the significant IxE we have detected in the observed data is not an artefact of the sample size and structure of our dataset.

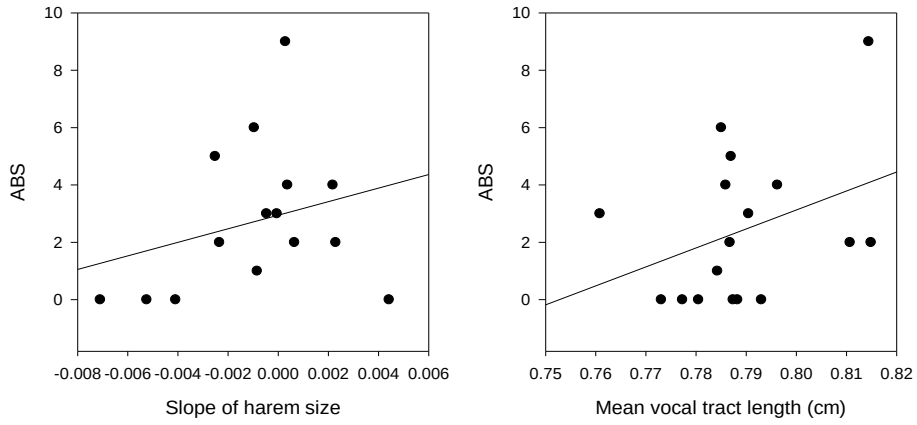
Selection

After model simplification, there was a weak suggestion for positive selection on males' slope of response in apparent VTL to changes in harem size, but this was not significant (Estimate=143.64±95.19, $F_{1,11=}$2.106, p=0.117, figure 4.5a). There was no evidence for selection on slope of response to the presence of an oestrous female, or to the slope of response to days since beginning rutting (see table 4.2). In the full model, there was no indication of a correlation between elevations in apparent VTL and annual breeding success (see table 4.2). However, because not all males were recorded in all contexts, although all males were assigned an annual breeding success, males which were not recorded in all contexts are excluded from the model, reducing the sample size. When analysing elevation without the slope terms in the model (thereby increasing the sample size) there was a non-significant positive correlation between apparent VTL and ABS (Estimate=64.29±44.59, $F_{1,13}$=2.08, p=0.173, figure 4.5b). It should be noted that this correlation is however therefore not independent of the effects of the slope of response to harem size.

Table 4.2: results of linear models investigating whether a correlation exists between annual breeding success and average apparent VTL (elevation), and annual breeding success and the slopes of response of males to changes in context. F values and p values are given for dropping each term from the full model.

Maximal model					
Term	Effect	S.E.	F value	d.f.	P value
Intercept	21.405	31.395			
Age	-2.271	1.378	2.376	1,3	0.263
Age^2	0.122	0.075	2.333	1,3	0.266
mean apparent VTL (cm)	-21.501	50.418	0.146	1,3	0.739
Slope of oestrous female	15.918	23.996	0.380	1,3	0.600
Slope of harem size	-193.502	268.323	0.414	1,3	0.586
Slope of days since began rutting	-76.900	130.274	0.279	1,3	0.650
Minimal Model					
Intercept	28.043	11.210			
Age	-4.776	2.281	4.385	1,11	0.060
Age^2	0.219	0.113	3.713	1,11	0.080
Slope of harem size	354.518	208.726	2.885	1,11	0.117

Figure 4.5: a) Correlation between annual breeding success (ABS) and male's slope of response of apparent VTL to changes in harem size, **b)** correlation between annual breeding success (ABS) and average apparent vocal tract length.



4.4 Discussion

To our knowledge, this is the first study to document inter-individual variation in acoustic BRNs in a wild population. Using a BRN framework to study variation in the apparent VTL of red deer males when roaring, we have found substantial variation between individuals in both elevation (average trait level) and slope of response to social context (presence of an oestrous female and harem size) and temporal context (time spent rutting). This therefore provides evidence that substantial inter- and intra-individual variation exists in the minimum formant frequencies of the roars of wild male red deer. Previous studies have suggested minimum formant frequencies represent honest signals of body size to conspecifics (Reby and McComb 2003a). Our findings suggest that in the wild, the information conveyed in this acoustic property of roars could vary with social context and internal state, and that both the magnitude and direction of motivation-dependence differs considerably between males, even over the course of a single breeding season (figure 4.4). Indeed, a substantial proportion (32%) of variation observed in apparent vocal tract lengths was explained by between-individual variation in behavioural reaction norms.

Although we found significant amounts of individual variation both for elevation and slope of response to context, the evolutionary implications of this variation are less clear. We found a non-significant trend towards a positive correlation between apparent VTL and annual breeding success. A previous study found significant evidence that average apparent VTL was related to breeding success in a study of 22 males (Reby and McComb 2003a); the contrast with our finding may result from the slightly smaller sample size, and/or because the previous study considered breeding success over multiple years. It should also be noted however that we use paternity data from a pedigree including genetic information, rather than behavioural data, in contrast to previous studies. We also found some limited evidence to suggest positive selection might be acting on the slope of response to harem size, indicating that males which increase their apparent VTL when defending larger harems gain higher fitness. However, there was no indication that males which responded more to changes in the presence of an oestrous female had higher fitness, or that males which could maintain the elevation of their apparent VTL over the duration of their rut were more successful.

While we have little evidence for selection on either elevation or slope, this does not necessarily indicate that the variation observed in apparent VTL has little evolutionary or

ecological relevance. The expected direction of the correlation between slope and fitness is not straightforward to predict, and a simple, linear correlation may be too simplistic an expectation. For example, individuals may be selected to be consistent in their response, rather than increasing or decreasing apparent VTL in response to a change in context. Despite the lack of evidence for selection on acoustic reaction norms, it is clear that ignoring between-individual variation in changes in traits over a breeding season can misinform our understanding of selection on those traits. Had we not considered this inter-individual variation in response, we would have concluded that the presence of an oestrous female, and a male's harem size, had no effect on the minimum formant frequencies produced in a male's roar. Botero *et al.* (2010) recently argued that single regression lines may not be appropriate for describing sender codes in natural populations, and that where variation between individuals is ignored, data is likely to be overdispersed.

In general however, the adaptive nature of the variation observed remains to be explained, and several potential avenues exist for future work. As an initial step, it would be illuminating to examine intra-individual consistency in apparent VTL over short time periods (*i.e.* within a recording session within each context level). Given the substantial within-individual consistency expected in this trait (Reby and McComb 2003) we would expect individuals to show little variation between roars when context, motivation or condition did not vary between samples; doing so would therefore clarify the role of measurement error and the relative magnitude of changes in apparent VTL between contexts. Variation in BRNs of apparent VTL may be related to variation in motivation and threat, as this could influence the appropriate response of the male to the context. We might expect males to extend their vocal tracts when guarding an oestrous female or a larger harem either to maximise the impression of body size to potential challengers or to make them potentially more attractive to females (Reby *et al*. 2005, Charlton *et al*. 2007). However, it has been shown that males challenged predominantly by young males increase VTL less than those challenged by other prime-aged males (Reby *et al* 2005) and so apparent extension may depend on the number of challengers, or the formant dispersion of the roar produced by the challenging male. Rank-dependent adjustment of acoustic characteristics has also been shown in humans: males who perceive themselves as dominant to their competitor lower the pitch of their voice when speaking to that competitor, whilst males who perceive themselves to be subordinate raise the pitch of their voice (Puts *et al.* 2006). In red deer, the cost-benefit ratio of engaging in a fight is likely to be dependent on current condition and motivation as well as body size, and so a

stag that has little to gain from fighting, or is in poor condition, may reduce the apparent extension of their vocal tract relative to their competitor, in order to appear subordinate and avoid a potentially lethal fight (Clutton-Brock *et al.* 1979). Further, errors in self-assessment may be important in maintaining individual differences in signalling (Botero *et al.* 2010); here such errors could arise from prior experiences which are not representative of the population (for example, encountering a non-random subset of the male population, see Whitehouse 1997). Finally, we have presented evidence of correlations between male's average VTL and their response to context, suggesting males may be constrained in their response (see below for discussion in relation to time spent rutting). Maximum VTL is known to be constrained by the skeleton (Reby and McComb 2003a); therefore males roaring at, or near, this maximal VTL are unlikely to be able to respond to being joined by an oestrous female, or an increase in harem size by increasing apparent VTL. This potential constraint therefore suggests multiple signalling strategies: signalling with high intensity which cannot be increased with changes in context, or signalling with low intensity but respond to change in context by increasing intensity.

Our evidence for inter-individual variation in changes in extension of vocal tracts over the rut (Figure 4.2c) contrasts with a recent study (Vannoni and McElligott 2009), in which fallow deer did not show changes in formant dispersion over the rut. These authors argue that this implies formant dispersion is less sensitive to changes in condition than vocalization rate, which has been shown to decrease in the later part of the season in both red and fallow deer (Clutton-Brock and Albon 1979; Vannoni and McElligott 2009). Fallow bucks roar at higher rates than red deer males, and do not fully extend their larynges to the sternum with each vocalization (McElligott *et al.* 2006), which may result in differences between the two species in the impact of changes in condition on formant dispersion. However, importantly, the authors did not measure inter-individual variation in changes in formant dispersion with condition, which, as noted above, can mask interesting within-individual patterns of variation. Although the costs associated with vocalizations are often negligible in warm-blooded animals (Reby and McComb 2003b), given the muscle activity required to extend the vocal tract and that males clearly often do not roar at full extension, it seems unlikely that there is no cost involved (although stags may trade-off roaring less frequently with maintaining vocal tract extension). The significant IxE we have found in male response of apparent VTL to time spent rutting suggests different males could be experiencing condition loss at different rates, perhaps related to initial condition, or the level of competition they have experienced. We found a

negative correlation between slope of response to time spent rutting and elevation of apparent VTL, indicating that males with the highest average VTLs showed the greatest decline over their rutting period (see figure 4.4c in comparison to figure 4.1e). This may be because males roaring with such extended vocal tracts can not extend them further, and so unlike males which had not maximally extended their VTL, were limited to remaining at their elevation or shortening it over time. Alternatively, the cost of roaring with such an extended vocal tract may result in males becoming more quickly exhausted and a faster decline in signal intensity over the rut.

Understanding how selection acts upon variation in acoustic and other signalling behaviours, and how such variation is maintained, remains a major challenge. A recent study has noted that formant frequencies have the potential to be imprecise, and that this could undermine their utility in sexual signalling (Sanvito *et al.* 2007). Here however, for the first time, we have shown how variation in formant frequencies could in fact encode for more precise information about motivation and condition. The significant levels of within-individual variation in acoustic structure of male roars, and their relation to context and condition, suggest that the formant frequencies of these vocalizations potentially encode short-term signals of motivation in addition to information on body size. Our results do not indicate that formant frequencies are not an honest indicator of body size when the larynx is fully lowered: if on average larger individuals roar with more closely spaced formant values, the honesty of the signal will be maintained. Indeed, the positive (but non-significant) correlation between annual breeding success and average apparent VTL suggests this is true. Instead, such within-individual variation in formant dispersion with condition and motivation potentially increases the honesty and relevance of vocal signals to interactions between males, because current condition and motivation are likely to be important determinants of current fighting ability. However, if females use formant dispersion to assess potential mates, as suggested in experimental studies (Charlton *et al*. 2007), the utility of such variable information when signalling to females is less clear. Although playback experiments in captive populations found oestrous females preferred males that have longer VTLs, in playbacks in the wild, peri-oestrous females paid more attention to roars simulating sub-adult males (Charlton *et al*., 2008). In general, the substantial variation we have observed in reaction norms for this acoustic cue suggests a complex interaction of motivation and threat may determine the signal a male produces. Collecting sufficient data from wild populations to identify such variation in acoustic behavioural reaction norms is often challenging; however, our results indicate that

demonstrating and understanding this variation is crucial to understanding the role of vocalizations in sexual behaviour, and more widely the maintenance of variation in behavioural traits.

Chapter 5:

Re-mating across years, intra-lineage polygyny and inbreeding

5.1 Summary

The interplay between philopatry and non-random mating has important consequences for the genetic structure of populations, increasing co-ancestry within social groups but also increasing the risk of inbreeding. Here, using genetic paternity data, we show that females in a wild population of red deer exhibit surprising mating behaviours which are associated with marked consequences for co-ancestry and inbreeding events in the population. Around a fifth of females mate with the same male in multiple years, and female relatives also frequently mate with the same male (intra-lineage polygyny); both of these behaviours occur more than expected by chance. Using simulations, we demonstrate that temporal and spatial factors are important in promoting both remating behaviours and intra-lineage polygyny, but are not sufficent to explain the extent to which they occur. Further, we show that re-mating and intra-lineage polygyny are associated with increased pairwise relatedness in the population, and also a rise in average individual inbreeding coefficients, with the latter particularly resulting from a non-random distribution of the rutting male population with respect to relatedness. Such mating behavours, and their consequences for the genetic structure of the population, are extremely rarely documented in wild polygynous mammals, yet have important implications for our understanding of social evolution, inbreeding avoidance and dispersal in such systems.

5.2 Introduction

In recent years, our understanding of mating systems has been revolutionised by the use of molecular techniques to assign parentage in wild populations (Hughes 1998), revealing hitherto unknown or unproven complexity, including alternative male strategies, extra-pair fertilizations and female choice/promiscuity (Coltman *et al.* 1999, McEachern *et al.* 2009, Twiss *et al.* 2006, Worthington-Wilmer *et al.* 2000, Griffith *et al.* 2002). Molecular techniques have also been revolutionary in revealing fine-scale spatial genetic structure arising from limited dispersal in a variety of wild vertebrate taxa (Piertney *et al.* 1999, Shorey *et al.* 2000, Nussey *et al.* 2005b*).* In polygynous mammals, it is most common for females to be philopatric and males to disperse, leading to aggregations of females in matrilineal groups (Greenwood 1980, Clutton-Brock 1989). Where a philopatric structure such as this is combined with non-random mating strategies, it can have substantial effects on kinship and inbreeding within groups (Chesser 1991).

Two female mating strategies have been revealed in polygynous mammals which, whilst currently extremely rarely reported, are potentially highly significant to the link between philopatry and co-ancestry/inbreeding. These are "mate fidelity", females mating with the same male in one or more distinct breeding attempts (termed 're-mating' in this study); and "intra-lineage polygyny", whereby female relatives show a propensity to mate with the same male (Rossiter *et al.* 2005). Females mating with the same male in multiple breeding seasons is an unexpected feature of polygynous mating systems in which females and males are spatially segregated outside of the breeding season, given it requires a re-association of the pair during the breeding season (in contrast to permanent pair bonds exhibited in monogamous species). Despite this, in a study of paternity in grey seals, *Halichoerus grypus*, 30% of maternal half-sibs were found to be full sibs, suggesting many females were re-mating with the same male across years (Amos *et al.* 1995). However, using a longer time series, the proportion of full sibs was later estimated to be substantially lower in the same population (Worthington-Wilmer *et al.* 2000), and no evidence of re-mating was found in a population of harbour seals (Coltman *et al.* 1998). Other evidence for re-mating has been found in the horseshoe bat, *Rhinolophus ferrumequinum*, (Rossiter *et al.* 2005), in which the authors showed 56.8% of females mating in more than one year paired with the same male in multiple years, and that such repeated pairings between individuals occurred more than expected by chance.

In the horseshoe bat, further to evidence for re-mating, the authors also found evidence that matrilineal relatives mated with the same males more frequently than expected by chance (termed "intra-lineage polygyny"). Intra-lineage polygyny is expected to arise when there is both strong philopatry amongst females - so that females are likely to associate in kin groups - and also strong polygyny, so that those groups of females are likely to be monopolized by single males. This interplay between philopatry and polygyny, resulting in intra-lineage polygyny, is likely to have important consequences for population genetic structure, increasing co-ancestry amongst females within social groups (Chesser 1991). Such effects have been implicated in social evolution, as raised co-ancestry, and therefore kinship, is likely to promote cooperative behaviours (Hamilton 1963, West *et al.* 2002, Griffin and West 2003). Indeed, in the horseshoe bat, intra-lineage polygyny combined with females repeatedly pairing with particular males was associated with an increase in pairwise relatedness coefficients, and significant genetic differentiation between groups of matrilineal relatives; the authors argued this was likely to strengthen ties between roosting females, and therefore promote cooperation within social groups (Rossiter *et al.* 2005). However, where generations of females are overlapping, intra-lineage polygyny and females re-mating with previous partners can also increase the potential for inbreeding to occur (Chesser 1991, Storz 1999). The extent to which these processes result in increased individual inbreeding coefficients will be dependent upon whether males show fidelity to mating sites between years, whether male tenure overlaps with the onset of sexual maturity of female offspring and whether there is random dispersal of male offspring, particularly whether male offspring ever obtain mating success within their natal group (Storz 1999). In general, the risk of inbreeding is not increased by female philopatry unless there is also a non-random spatial distribution of males with respect to relatedness (Foerster *et al.* 2006). Further, even where demographic circumstances increase the potential for inbreeding, if individuals are able to recognise kin, they may avoid mating with them (Pusey and Wolf 1999, Foerster *et al.* 2006). In the horseshoe bats, no increase in inbreeding was found from that expected under random mating (Rossiter *et al.* 2005).

In this study, we use molecular paternity data to examine patterns of mating in a wild population of red deer, quantifying the extent to which females mate with the same male in multiple years, and to which females from the same matriline tend to mate with the same male. Further, we examine associated changes in pair-wise relatedness and the number of inbreeding events within the population. Testing whether females re-mate with

previous partners, for the presence of intra-lineage polygyny and for the effects of such parameters on relatedness and inbreeding coefficients necessarily requires comparing the observed mating outcomes with those expected under random mating, which can be calculated using simulated data. Such techniques can also be used to determine whether the observed outcomes are a product of the breeding system, such as a preference for particular mating sites, or a specific mating strategy. This method of pedigree simulation, incorporating assumptions about mate availability and spatial parameters, has been successfully used to assess whether inbreeding avoidance occurs more often than expected under random mating (Keller and Arcese 1998, Hansson *et al.* 2006, Szulkin *et al.* 2009); yet to date studies examining pairs re-mating have relied on somewhat anecdotal evidence to suggest the findings are not an outcome of site fidelity (Amos *et al.* 1995, Rossiter *et al.* 2005). Although the effects of non-random mating on genetic structuring are not contingent on the mechanisms underlying it, using simulations in this way to get at the proximate cause can shed light on the processes involved: for example, whether female choice or non-random male dispersal is likely to play an important role.

This study: the potential for re-mating and intra-lineage polygyny

In this study we assess i) how commonly mating pairs re-mate in subsequent years, and ii) the extent to which members of the same matriline mate with the same male, in a population of wild red deer, *Cervus elaphus*, living on the North Block of the Isle of Rum, Scotland. We also examine concomitant changes in inbreeding risk and relatedness coefficients. Red deer have a polygynous, harem defence mating system, in which males compete to herd and defend groups of females, and to mate with females within those groups which are in oestrus. Various spatial and temporal aspects of the mating system suggest the potential for both re-mating and intra-lineage polygyny. Males live outside the study area for the majority of the year, returning prior to the breeding season (rut) to the main hind feeding grounds to mate. Young males disperse from their natal groups after the age of 2, and outwith the rut, adult males do not show spatial genetic structure (Clutton-Brock *et al.* 1982, Nussey *et al.* 2005b). However, whether there is spatial genetic structuring of males during the rut, when they return to defend harems in the study area, is unknown. Preliminary analyses have suggested a male's location during the rut is highly repeatable, with 50-70% of variance in male location explained by male identity (Stopher *et al. unpublished data*), implying males return to rut in the same area in multiple years. Females in this population are philopatric, usually remaining within the natal group

to which they were born, so that the female population consists of mostly matrilineal groups which demonstrate strong location fidelity (Albon *et al.* 1992). Very fine-scale genetic structuring (<100m) has been shown amongst females (although this has declined over time, Nussey *et al.* 2005b). During the rut, females occupy a constricted version of their normal home range (Clutton-Brock *et al.* 1982). Overall, therefore, the potential for males and females to mate in the same location each year is high, as is the potential for female relatives to be mating in the same place.

There is also substantial individual consistency of rut timing. Males generally do not rut for the entire breeding season, but at some point become exhausted and leave the rutting area; male rut start, median and end dates have been shown to be highly repeatable within individuals (Clements *et al.* 2010). Females are in oestrus only briefly, and usually mate only once (Clutton-Brock *et al.* 1982). Although the majority of oestruses occur during a two week peak of the breeding season, they can be dispersed over up to 4 months. Female oestrus date has not been found to be particularly repeatable within individuals; however, this finding is potentially confounded by the power available to detect repeatability, as parturition date is highly repeatable and the two are significantly correlated at both the phenotypic and genetic level (Clements *et al.* 2010). Non-lactating females which are closely associated within the same social group have been found to have synchronised oestruses (Iason and Guinness 1985), and further, Clements *et al.* (2010) noted a significant sire effect on female oestrus date, suggesting there may be consistent spatial differences in female oestrus date combined with fidelity of rutting sites by males across years. This therefore suggests female relatives associating within the same area or matrilineal group may be likely to be in oestrus at the same time, and are therefore more likely to mate with the same male.

In this study, we compare the observed mating outcomes of the annual red deer rut, derived from a genetic pedigree, to those produced under a number of random mating scenarios, each with sequentially greater constraints. These were: fully random mating, temporally constrained mating, temporally and spatially constrained mating, and finally temporally and spatially constrained in which the probability of a male mating is dependent upon age. We compare the frequency at which repeated pairings occur and the levels of intra-lineage polygyny, in the observed and simulated pedigrees, as well as relatedness and individual inbreeding coefficients, to determine the extent to which such

non-random mating occurs and the effect it has on the relatedness structure of the population.

5.3 Methods

Study system

Data was collected from a wild population of red deer, *Cervus elaphus*, resident in the North Block of the Isle of Rum, Scotland, which have been intensively studied since 1971. The study area comprises approximately 14% of the island area as a whole, and between 15-25% of the deer on the island. In this study, we studied mating success during the ruts of 1971-2006. In this population, all individuals can be recognised, either through natural markings or artificial markings applied when individuals are captured at birth. Matrilines were assigned to individuals using their oldest known female relative. 85 matrilines exist, with a maximum of 9 generations over the years used in this analysis. Few females immigrate into the population; therefore the majority (73) of the matrilines originate from 1974 or earlier. During the rut, daily censuses are conducted which record the location (to the nearest 100m) and identity of all females, and all males which are defending harems of females. Female oestrus date can be calculated by backdating from the date of birth of subsequent offspring by 235±5 days; we then assume that the female has conceived within this eleven day 'oestrus window' (Clutton-Brock *et al.* 1982). Females produce one offspring per year, although not all females breed in each year. Females can conceive at the age of two; after the age of five female fecundity is generally constant until it begins to decline at around 13 years (Nussey *et al.* 2009). Male annual breeding success is highly skewed (Clutton-Brock *et al.* 1982) and is strongly correlated with age (Nussey *et al.* 2009). Males rarely breed before 5, with ABS peaking at 8-10 years and then declining in later life (Nussey *et al.* 2009). Males therefore begin breeding much later in life and have a much shorter breeding tenure than females.

Paternity Assignment

Daily observations are made during the calving season (approximately 20th May to 30th June) to identify calving date for each female and monitor neonatal survival (Clutton-Brock et al. 1982), and to catch calves and take tissue samples for genotyping. Other individuals not caught at birth are sampled from cast antlers, by chemical immobilization

or post-mortem. Individuals born since 1991 were genotyped at up to 15 highly variable microsatellites; prior to this individuals were genotyped at up to 8 microsatellites. Paternities were assigned using the programs MasterBayes (Hadfield et al. 2006) and COLONY2 (Wang and Santure 2009) with greater than 80% individual confidence, with preference given to assignments made by the MasterBayes program, and COLONY2 used to assign paternities where MasterBayes could not assign a father at 80% individual confidence (see Walling et al. 2010 for full details). The use of categorical pedigrees such as this in our analysis is potentially misleading, as categorical pedigrees do not explicitly incorporate the error around paternity assignments. Analysis was undertaken to address this potential problem (presented in Appendix A) and we were able to demonstrate it has no effect on our findings.

Analysis

All analyses were carried out in R 2.8.1 (R Development Core Team 2008).

Randomisations

For each year, lists of candidate females (those which calved the following spring) and candidate males (those seen to hold a harem in that year) were drawn up, and five sets of simulated pedigrees were generated:

1. 'Random': each female was randomly assigned a male from the candidate male list.

2. 'Temporal Random': each female was randomly assigned a male from the candidate male list that was known to have held a harem during her calculated 'oestrus window'.

3. 'Spatial Random': as for temporal random, but the list of potential males was further restricted to those holding a harem within a) 500m ("Spatial 500m") or b) 100m ("Spatial 100m") of the female's location on the potential day of conception. These values were chosen after preliminary analysis revealed that 75% of females mate with males rutting within 500m of their location on the day of conception, and 50% of females mate with a male within 100m of their location.

4. "Age corrected": as for "Spatial 100m"; but with the sampling of temporally and spatially available males weighted by the probability of males gaining reproductive success given their age. We constructed a linear model of age and its quadratic term against male annual breeding success for the pedigree data used in this study (2083 observations across 603 males) and from this extracted the probability of males of different ages gaining a paternity. The sampling of candidate males was then weighted by this probability.

Although matings between candidate males and females were necessarily conducted on an annual basis, for each randomisation type these randomisations were combined so as to maintain the temporal structure of that pedigree for that randomisation type and iteration (such that, for example, a mother-son relationship within $year_t$ was maintained in $year_{t+i}$) Each randomisation was constructed on an annual basis, but then for each randomisation type all years were combined to produce a randomised pedigree covering the whole study period. This was repeated 1000 times for each randomisation type. Only calves for whom a paternity could be assigned in the observed pedigree were assigned a father in the randomised pedigrees, as a higher rate of paternity assignment rate in the randomisations relative to observed pedigree would undermine comparisons of re-mating, intra-lineage polygyny, relatedness and inbreeding.

Pedigree statistics

Pedigree statistics (*e.g.* re-mating frequency, intra-lineage polygyny, pair-wise relatedness and inbreeding coefficient) were calculated for each of the 1000 simulations of each randomisation type, and then an average taken of these statistics; therefore note that reported standard deviations are the standard deviation around that average.

All measures described were compared between the observed pedigree and the average of the 1000 simulations for each randomisation type, using Z tests with the standard deviation as described. Given a large number of Z tests were carried out (50), we used a Bonferroni correction to calculate the appropriate significance level as $p<0.001$.

1. Calculating frequency of re-mating

For each male-female pair known to have mated, we calculated whether they had re-mated when they had the opportunity to do so. This gave us the number of pairs, number of females and number of males which did re-mate and the number which did not, despite having the potential to do so. The opportunity to re-mate is restricted by the presence or absence of previous partners. In addition to deaths and births changing the available populations of females and males over the study period, in calculating opportunities to mate we also took into account that i) females do not conceive every year and ii) most males spend the majority of their time resident outside of the study area, only returning for the rut, and not all males known to be alive in the study population return each year. Therefore, for any pair which had mated, we calculated in which other years both i) the female of the pair was receptive to mating (conceived and gave birth to a calf the following year) and ii) the male rutted within the study area (and was therefore a potential father in the paternity analysis), and scored whether they re-mated in that year (1/0).

From this, we then calculated the number of pairs which had mated in more than one year divided by the number of all pairs known to have the opportunity to mate in more than one year (as a percentage). We then calculated the percentage of females and males in known pairs which were involved in re-mating events. We also calculated a number of other statistics describing patterns of re-mating: i) the average size of full sib-ships within the pedigree and ii) the ratio of unique males a female mated with in her lifetime to the number of offspring she produced.

2. Calculating the extent to which female relatives mated with the same male (intra-lineage polygyny)

We calculated the ratio of unique females a male mated with in his lifetime to the number of unique matrilines those females came from; so that a value of one describes a male who never mated with females which were relatives, and values less than one indicate increasing amounts of intra-lineage polygyny.

3. Relatedness and inbreeding coefficients

Pair-wise relatedness coefficients were calculated using the R package "kinship" (Atkinson 2008). Inbreeding coefficients were calculated using the R package "pedigree" (Coster 2008): we calculated average coefficients, the total number of non zero coefficients and the number of coefficients greater or equal to 0.125 (representing close inbreeding events).

Genetic Structuring of the rutting male population

Pairwise relatedness coefficients were calculated for all males in the pedigree using the "kinship" package (Atkinson 2008). To calculate spatial distances between males, we calculated the lifetime average rutting location of each male to the nearest 100m from census data, and from this calculated distances between these locations for each pair of males in metres. The correlation between pair-wise relatedness and pair-wise spatial separation was tested in a linear mixed effects model, with relatedness as the response variable and the identity of each of the pair as random effects.

5.3 Results

Re-mating frequency

9.20% of pairs mated in more than one year, and 22.43% of females and 25.86% of males mated with a partner with whom they had mated previously (see table 5.1, and figure 5.1 for an example of this). This was significantly higher than expected under either random mating ("Random"), random mating constrained to males rutting when a female was in her oestrus window ("Temporal"), and random mating constrained to males rutting within 500m of a female during her oestrus window ("Spatial 500m", see table 5.1, figure 5.2). The percentage of pairs and males re-mating was also significantly higher in the observed pedigree than in the "Spatial 100m" or "Age-Corrected" simulations (see table 5.1). However, after a Bonferroni correction, the observed percentage of females re-mating was marginally non-significantly greater in than in the "Spatial 100m" simulation, and was not significantly greater than in the "Age Corrected" simulation ("Spatial 100m", $Z=3.07$, $p=0.001$, "Age Corrected", $Z=2.68$, $p=0.004$, Bonferroni level of significance=0.001).

Considering only calves with assigned paternity, on average, females bred in 3.32±0.11 years, with 3.00±0.09 unique males. In total, 134 parental combinations, made up of 108

females and 60 males were repeated on an average of 1.15±0.04 occasions. Most re-matings occurred only once; however four pairs re-mated 3 times (i.e. mated four times). Re-mating events generally occurred in consecutive years; but some occurred as much as five years after the original mating. As a consequence of the observed re-mating, full sibship sizes were significantly higher in the observed pedigree than in any of the simulated pedigrees (see table 5.2). The ratio of unique males a female mated with to calves produced was also significantly lower in the observed pedigree than in any simulated pedigree (see table 5.2, figure 5.3): this again is an expected consequence of pairs re-mating.

Figure 5.1: Pedigree illustrating pairs re-mating and intra-lineage polygyny in matriline 153. Squares refer to males, circles to females, and triangles to other offspring not of interest here. The two males shown, "AIDAN" and "SIMPL" can be differentiated by colour. "SIMPL" was involved in several re-mating events, including mating with two females (marked †) in three breeding seasons. "AIDAN" sired both starred offspring; this increased their relatedness coefficient from that of aunt-half niece (0.125) to aunt-half niece and full sibs (r: 0.125+0.250=0.375).

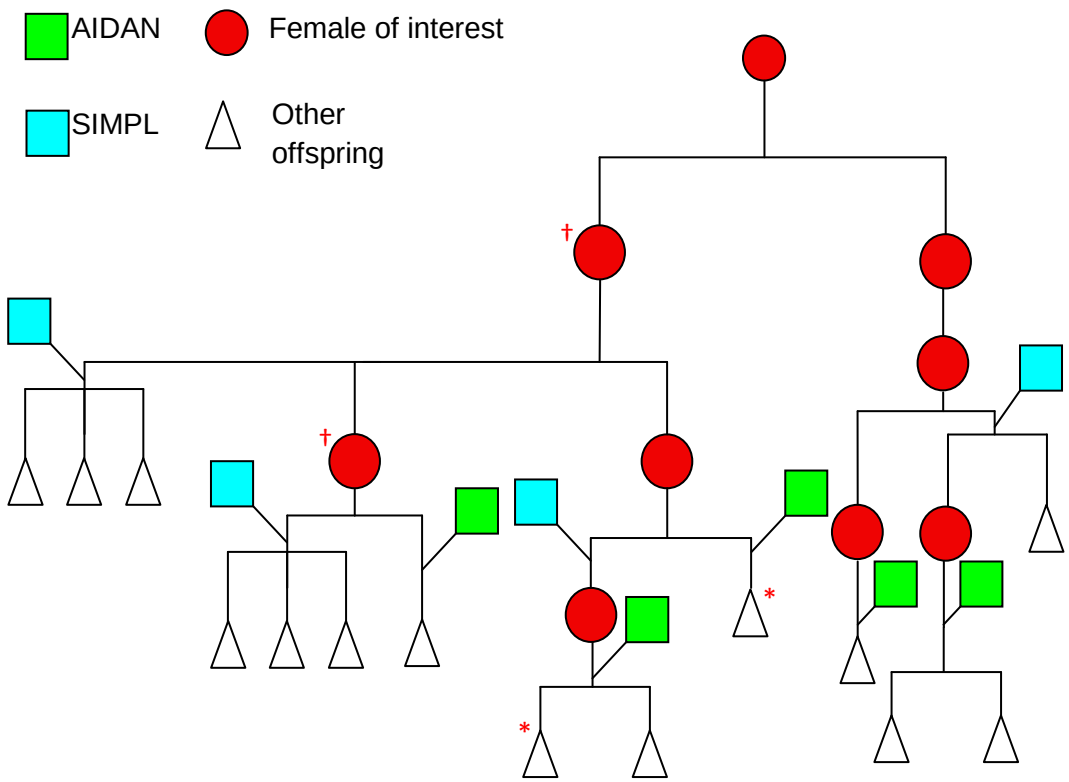


Figure 5.2 Percentage of pairs, females and males which were involved in at least one re-mating event, for the observed dataset and in the simulated pedigrees.

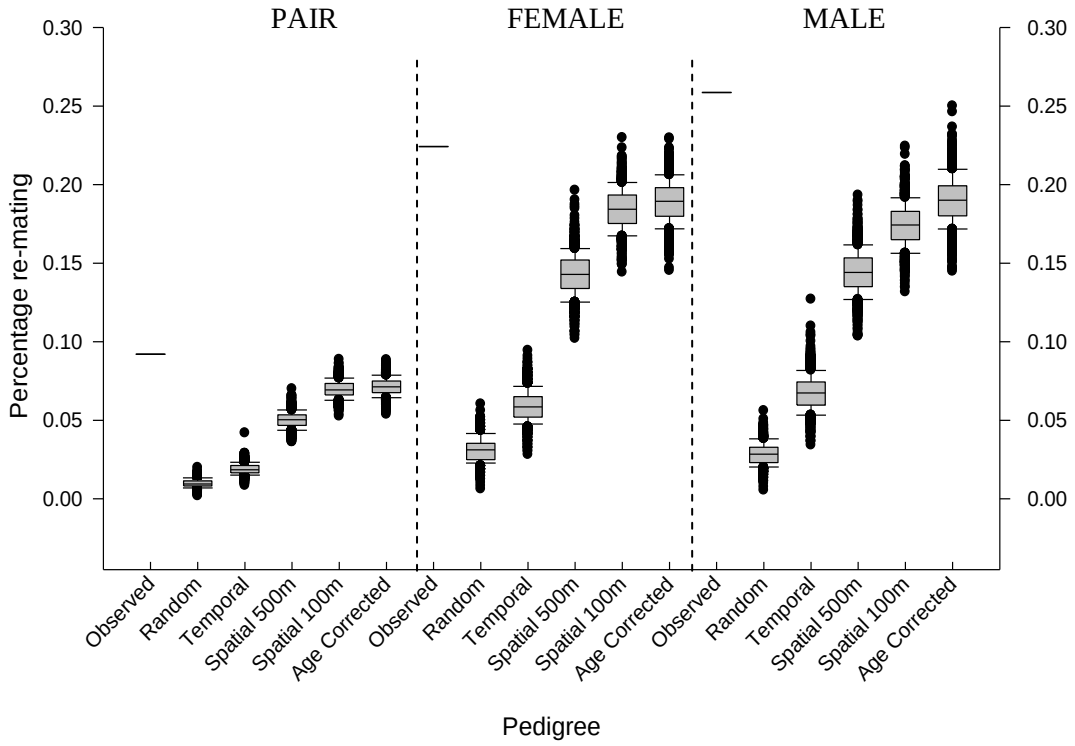


Figure 5.3: frequency histogram of the ratio of unique males a female mated with to the number of offspring the female produced, in the observed population and simulated pedigrees. For simulations, an average of the 1000 runs was taken.

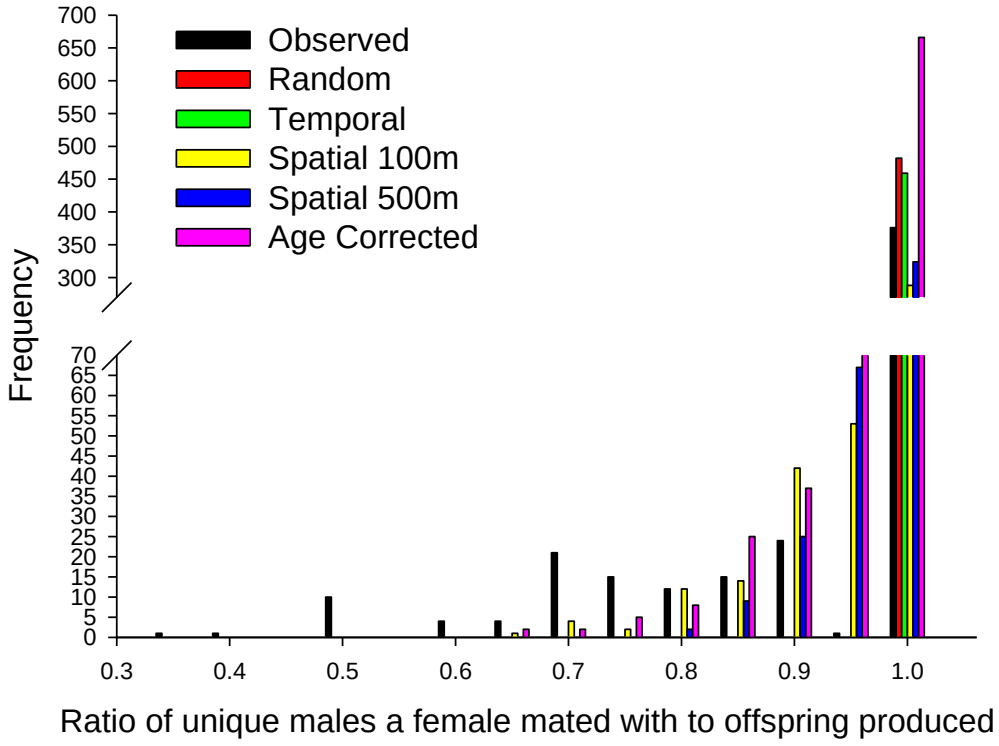


Table 5.1 Re-mating frequency in observed pedigree and in randomisations, and comparison. Standard deviations given are for the distribution of percentages from the 1000 runs of the pedigree simulations. Z values and p values are given for a one-tailed test of the hypothesis that population level re-mating frequency is significantly higher than would be expected in each randomisation. The percentages themselves vary between males, females and pairs because of the different totals of each category in the denominator of the calculation: there are more pairs in total, and fewer males than females.

Model	% Pairs Re-mating	SD	Comparison to observed re-mating frequency		% Females Re-mating	SD	Comparison to observed re-mating frequency		% Males Re-mating	SD	Comparison to observed re-mating frequency	
			z	P			z	P			z	P
Observed	**9.20**		N/A	N/A	**22.43**		N/A	N/A	**25.86**		N/A	N/A
Full random	**0.98**	0.25	33.4	<0.0001	**2.84**	6.97	28.11	<0.0001	**2.85**	0.70	33.04	<0.0001
Temporal random	**1.89**	0.33	22.0	<0.0001	**5.93**	9.93	16.61	<0.0001	**6.74**	1.13	16.95	<0.0001
Spatial random (100m)	**6.97**	0.54	4.1	<0.0001	**18.44**	1.30	3.07	0.001	**17.43**	1.40	6.04	<0.0001
Spatial random (500m)	**5.02**	0.51	8.2	<0.0001	**14.26**	1.33	6.12	<0.0001	**14.41**	1.40	8.16	<0.0001
Age Corrected	**7.14**	0.55	3.7	<0.0001	**18.90**	1.32	2.68	0.004	**19.01**	1.48	4.61	<0.0001

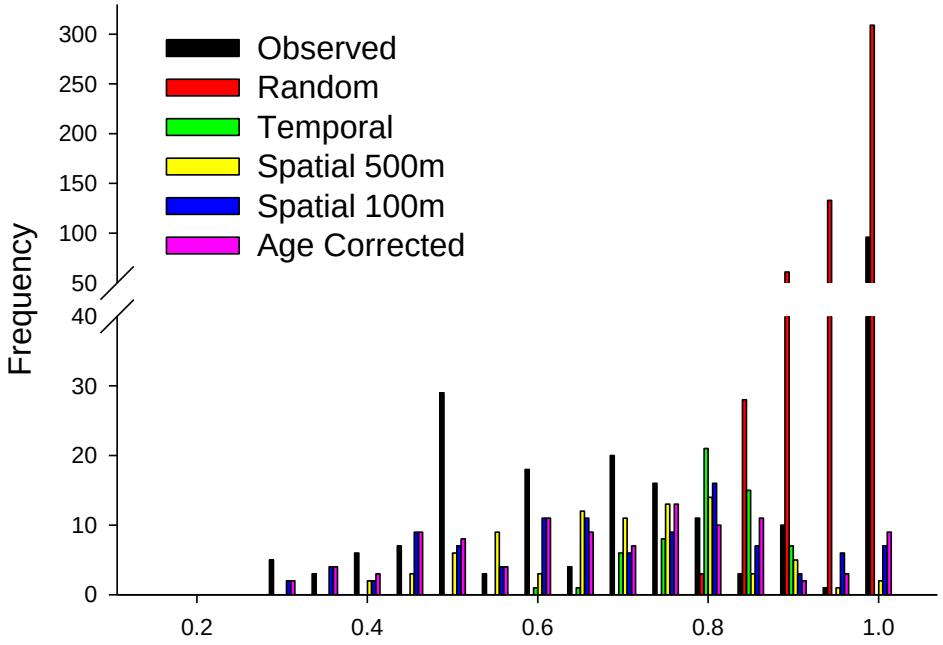
Table 5.2 A comparison of various statistics describing the pedigree for the observed and each randomized pedigree: full sibship sizes, the ratio of unique males a female mated with over her lifetime to the number of offspring she produced, and the ratio of unique females a male mated with to the number of unique matrilines he mated with (intra lineage polygyny). For randomized pedigrees, averages are given- i.e. the average of the statistic over the 1000 runs of the pedigree simulation, and the standard deviation of the distribution of those averages, rather than the standard deviations of the statistic for each pedigree simulation run (hence no standard deviation is given for the observed value). Z test and p values are given for a one-tailed test of the hypothesis that a) full sibship size is higher in the observed pedigree than the simulated pedigrees, b) the ratio of unique males a female mated with over her lifetime to the number of offspring she produced is smaller in the observed pedigree than the simulated pedigrees and c) the ratio of unique females a male mated with to the number of unique matrilines those males mated with is smaller in the observed pedigree than the simulated pedigrees.

Model	(Average) full sibship Size	SD	Comparison to observed		(Average) ratio unique males to offspring produced	SD	Comparison to observed		(Average) ratio unique females to unique matrilines	SD	Comparison to observed	
			z	P			z	P			z	P
Observed	1.106				0.941				0.778			
Full random	1.010	0.003	38.1	<0.0001	0.994	0.002	31.2	<0.0001	0.945	0.004	37.1	<0.0001
Temporal random	1.019	0.003	26.2	<0.0001	0.989	0.002	28.1	<0.0001	0.920	0.005	27.4	<0.0001
Spatial random (100m)	1.077	0.006	4.8	<0.0001	0.956	0.004	40.8	<0.0001	0.837	0.007	8.1	<0.0001
Spatial random (500m)	1.054	0.006	9.2	<0.0001	0.969	0.004	4.1	<0.0001	0.858	0.007	11.9	<0.0001
Age Corrected	1.078	0.006	4.4	<0.0001	0.954	0.004	3.4	0.0004	0.823	0.008	5.85	<0.0001

Intra-lineage polygyny

Males mated with females from the same matriline significantly more in the observed population than expected from any of the simulated pedigrees: the ratio of unique females a male mated with to the number of unique matrilines those females belonged to was significantly lower in the observed pedigree (see table. 5.2, figure 5.4 and see figure 5.1 for an example) than in any simulated pedigree.

Figure 5.4: Frequency histogram of the ratio of the number of unique matrilines to which a male's mates belong, to the number of unique females the male mated with, in the observed population and simulated pedigrees. Low values therefore indicate more extreme intra lineage polygyny. For simulations, an average of the 1000 runs was taken.



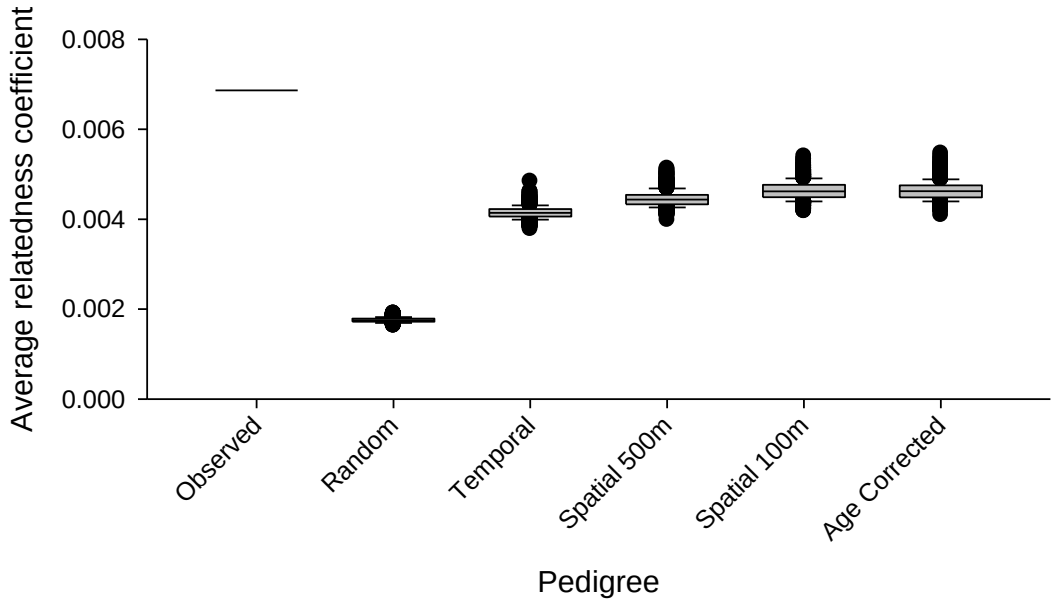
Relatedness

On average, pairs of individuals in the observed pedigree were significantly more related than expected under random mating: pairwise relatedness was significantly higher in the observed pedigree than under any simulation (see table 5.3, figure 5.5). Figure 5.1 illustrates how relatedness can be increased as a result of intra-lineage polygyny (see also discussion).

Table 5.3: comparison of pair-wise relatedness amongst individuals for observed and each simulated pedigree type. For simulations, the average value for the 1000 iterations of the simulation is given, with standard deviation. Z tests are presented for a one-tailed test that the observed value is significantly greater than the distribution of simulated values.

Model	(Average) relatedness	SD	Comparison to observed	
			z	P
Observed	0.00687			
Full random	0.00174	0.00005	102.6	<0.0001
Temporal random	0.00415	0.00013	20.9	<0.0001
Spatial random (500m)	0.00445	0.00017	14.2	<0.0001
Spatial random (100m)	0.00464	0.00020	11.2	<0.0001
Age Corrected	0.00463	0.00020	11.2	<0.0001

Figure 5.5: comparison of average pair-wise relatedness coefficients between individuals in the observed pedigree and in the simulated pedigrees.



Inbreeding

Average inbreeding coefficients were significantly higher in the observed pedigree than in any simulated pedigree (see table 5.4, figure 5.6). In addition, the total number of non-

zero inbreeding coefficients was significantly higher in the observed pedigree than in any of the simulations (Table 5.4). We inspected whether this effect was driven by close inbreeding events by determining whether it remained on considering only highly inbred individuals ($f \geq 0.125$), but it did not: the observed pedigree did not have significantly more close inbreeding events than in either the "Spatial 100m" or "Spatial 500m" simulations and the differences between the observed pedigree and the "Temporal" and "Age Corrected" simulations in the number of close inbreeding events were not significant after Bonferroni correction (see table 5.4, Bonferroni significance level p=0.001). This suggests that in the observed pedigree, the increase in average inbreeding coefficients and total number of inbreeding events compared to the simulations resulted from more deep inbreeding events occurring than expected, rather than more close inbreeding events. One route by which additional inbreeding events occur within a matriline is through intra-lineage polygyny, exemplified by the pedigree in figure 5.7.

Figure 5.6: comparison of inbreeding coefficients in observed pedigree, and the average for each simulated pedigree. Inbreeding coefficients are binned into groups representing key inbreeding events; however it should be noted that many inbreeding coefficients were values intermediate between these bins, due to the effects of intra-lineage polygyny (*e.g.* see Figure 5.7).

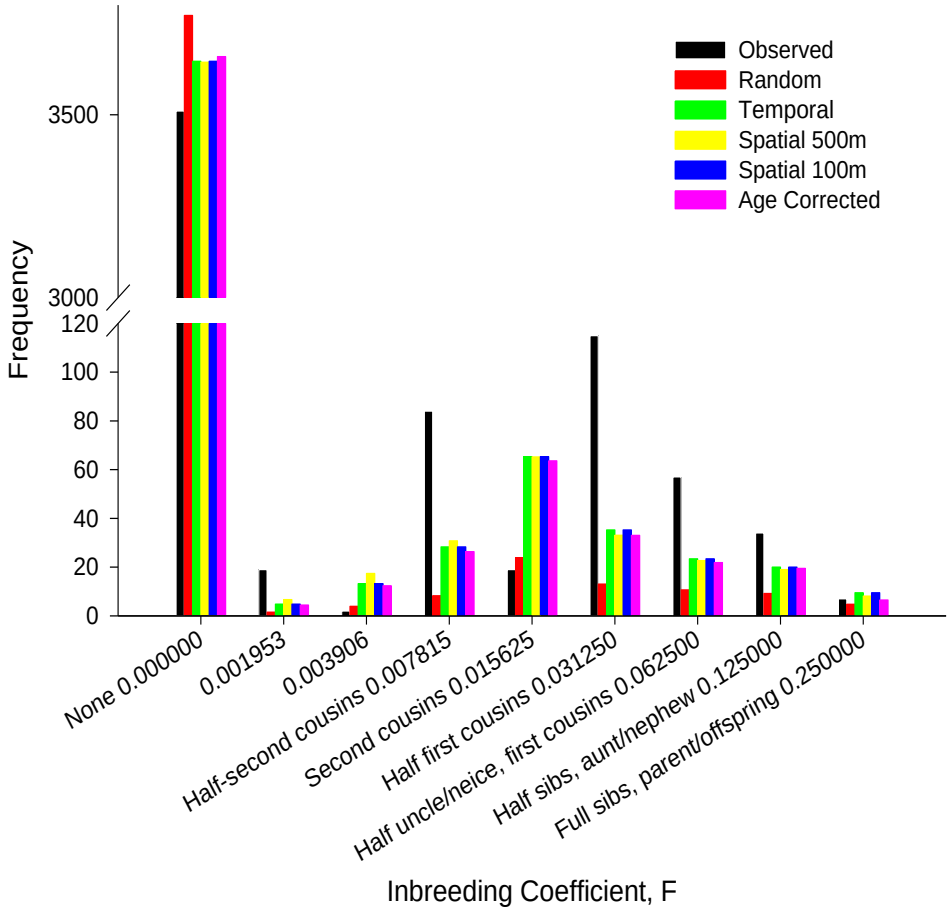
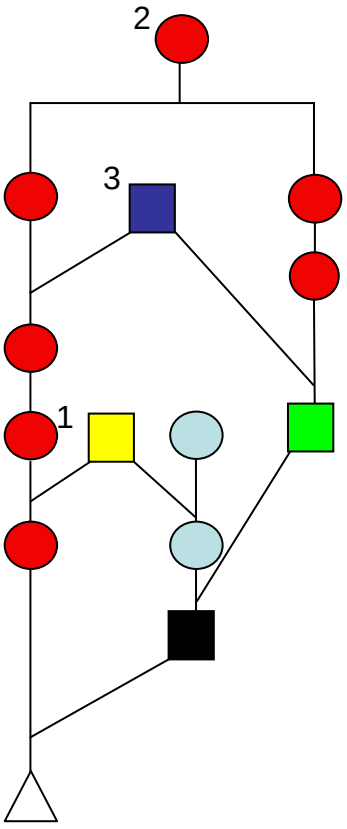


Table 5.4: a comparison of inbreeding statistics for the observed and each randomized pedigree: the average inbreeding coefficient, the number of non-zero inbreeding coefficients and the number of coefficients greater than or equal to 0.125. For randomized pedigrees, averages are given- i.e. the average of the statistic over the 1000 runs of the pedigree simulation, and the standard deviation of the distribution of those averages, rather than the standard deviations of the statistic for each pedigree simulation run (hence no standard deviation is given for the observed value). Z test and p values are given for a one-tailed test of the hypothesis that the statistic is higher in the observed pedigree than the simulated pedigrees. Inspection of close inbreeding events in the "Temporal", "Spatial 100m" and "Spatial 500m" simulations revealed that many of the close inbreeding events in these simulations consisted of mating events between mothers and sons, or half sibs, in which the males were less than 5 years old. These matings, whilst possible, are unlikely, and probably the result of immature males not yet having fully dispersed from the natal group. Therefore these estimates of close inbreeding coefficients in these simulations are inflated by these unlikely pairings, and so the comparison with the observed pedigree is a conservative one.

Model	**Average Inbreeding Coefficient**	SD	Comparison to observed		**(Average) Number of non-zero coefficients**	SD	Comparison to observed		**(Average) Number of coefficients >=0.125**	SD	Comparison to observed	
			z	P			z	P			z	P
Observed	**0.00304**				**339.00**				**32.00**			
Full random	**0.00094**	0.00018	11.4	<0.0001	**75.77**	11.61	22.7	<0.0001	**13.01**	3.69	5.2	<0.001
Temporal random	**0.00169**	0.00022	6.1	<0.0001	**217.57**	20.09	6.0	<0.0001	**20.06**	4.56	2.6	0.004
Spatial random (100m)	**0.00204**	0.00026	3.9	<0.0001	**202.82**	22.92	5.8	<0.0001	**26.43**	4.74	1.2	0.120
Spatial random (500m)	**0.00187**	0.00025	4.7	<0.0001	**206.21**	21.63	6.1	<0.0001	**23.81**	4.78	1.7	0.121
Age Corrected	**0.00177**	0.00023	5.5	<0.0001	**189.71**	21.40	7.0	<0.0001	**22.89**	4.60	2.0	0.024

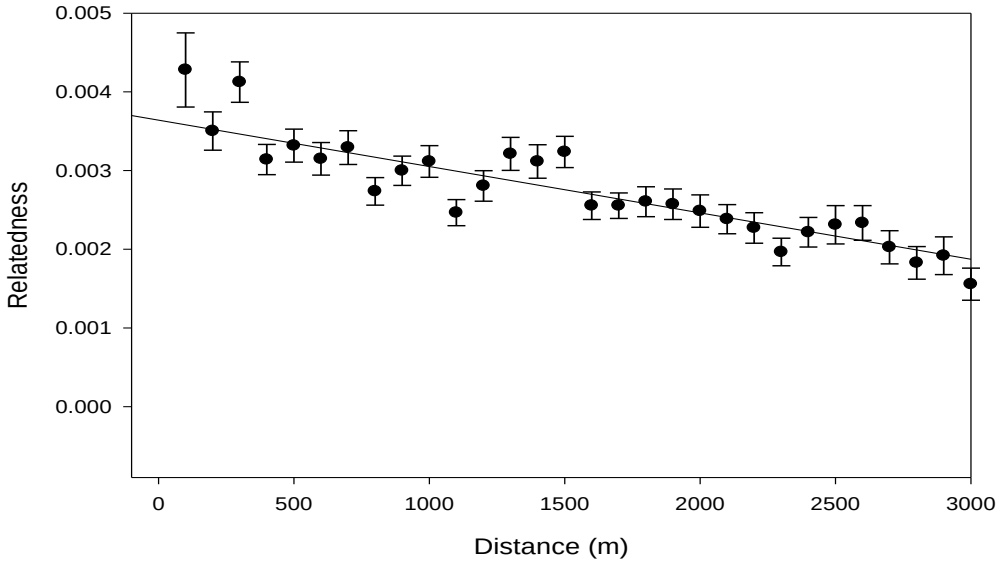
Figure 5.7: pedigree illustrating how intra-lineage polygyny increases inbreeding coefficients within a matriline. Squares represent males, with different colours representing different males. Females are represented by circles, and the offspring whose inbreeding coefficient is to be calculated is represented as the white triangle. The parents of this offspring are not only aunt-half nephew (loop 1) and half third-cousins (loop 2); but also, because an aunt and her half niece both mated with the blue male (loop 3, intra-lineage polygyny), half first cousins once removed. Therefore, the inbreeding coefficient for this individual is 0.0625+0.0019525+0.015625=0.08008.



Genetic Structuring of the Male Population

We found that the location of rutting males was non-random with respect to relatedness, so that more closely related males were more likely to rut in the same location: there was a significant negative correlation between male pair-wise relatedness coefficients and the pair-wise spatial separation (Effect=-1664, $F_{1,174434.4}$=159.23 p<0.001. Variance explained by first male identity=217471±12687 and by second male identity= 119852±4583, see figure 5.8).

Figure 5.8: relatedness of pairs of males plotted against the distance between their average rut locations (pair-wise spatial separation).



5.4 Discussion

In this study, we have demonstrated that a fifth of females mate with the same male across multiple breeding attempts, and that members of the same matriline frequently mate with the same male; in both cases these events happen more than would be expected under random mating. These mating behaviours are associated with a general increase in relatedness across the population compared to that expected under random mating, and are also associated with an increase in inbreeding events. It should be noted that, like nearly all wild studies, our estimates of inbreeding are likely to be conservative, given the assumption that founders and immigrants are unrelated, and because we cannot assign paternity to all individuals. Whilst this is also true for the simulated mating scenarios, and the validity of our conclusions is therefore maintained, individual inbreeding coefficients in this population are likely to be even greater than described in this study.

Levels of re-mating and intra-lineage polygyny observed in the population, and the consequent increase in relatedness and number of inbreeding events, were greater than expected from the simulated pedigrees in the majority of analyses. The percentage of pairs re-mating, the number of males re-mating with the same female, full sib-ship sizes, the ratio of unique mates to offspring produced, the extent of intra-lineage polygyny, average relatedness coefficients and average inbreeding coefficients were all significantly different in the observed data to the simulated pedigrees. In each case, a sequential improvement in

"fit" to the observed data was observed as more complexity was incorporated into the simulated pedigrees, so that the simulation incorporating temporal constraints to mating, spatial constraints to mating and an age-adjusted probability of males gaining paternity success ("Age Corrected") most closely predicted observed values in all but one analysis (relatedness, in which the "Spatial 100m" was closest to the observed value, but very similar to "Age Corrected"). This suggests that all of these constraints contribute to the extent of re-mating and intra-lineage polygyny which we have observed: within-individual, and within-matriline, consistency of individuals in their timing and location of rutting behaviour is likely to be important in facilitating re-mating between pairs and probably also intra-lineage polygyny across years. The further improvement upon adding in age-weighted reproductive probabilities for males probably results because most re-mating events happen in consecutive years, and males have a peak of reproduction which lasts for around three years; therefore, if males return to the same rutting locations, the male was likely to have been dominant in that area for two consecutive years. A similar argument can be applied to intra-lineage polygyny, in that female relatives mating in the same place in consecutive years will be likely to mate with the same male; further, within a year, female relatives may be more likely to mate with the same male because he is the most dominant male in the vicinity.

Although there are plausible biological explanations as to why each of the constraints incorporated into the simulations could explain the increase in re-mating and intra-lineage polygyny, it is worth considering other explanations for the concomitant increase in inbreeding events. In particular, any constraint applied to random mating is likely to increase variance in male mating success, and therefore result in a decrease in effective population size. Smaller effective population sizes are associated with increased risk of inbreeding (Wright 1984); therefore, the act of constraining random mating in itself could generate a change in the average inbreeding coefficient. This could be tested by comparing the variance in male mating success across the simulations, or by undertaking a further simulation in which a female's choice of mates was limited, but to a random subset of males.

However, regardless of the explanation for the improved fit of the simulated data to the observed data as simulated mating was constrained, it is important to note that significant differences still remained between simulations and the observed pedigree for most parameters, indicating the simulations did not capture the full extent of re-mating and

intra-lineage polygyny. It therefore seems likely that processes not captured by these simulations are also enabling these behaviours. Although for females, it seems all re-mating events are a consequence of temporal, spatial and male age or dominance constraints, the simulations did not capture all the processes driving males to re-mate with the same females, and therefore did not capture the proportion of pairs that re-mated. It is quite likely that re-mating in males was not fully captured by the simulations because in general, more successful males would be more likely to re-mate with the same female (see Appendix B). Although this is captured to some effect in the 'Age Corrected' scenario, factors other than age influence male mating success, such as body size, antler size and fighting ability (Clutton-Brock *et al.* 1979). Other factors which may affect variation in re-mating frequency are discussed in Appendix B.

No simulation fully captured the observed levels of intra-lineage polygyny. Female relatives may be more likely to mate with the same male than explained by spatial, temporal or male age factors because of mate copying; or simply because the close association of female relatives means they are likely to be found in the same harem. Although this will be captured to some extent in "Spatial 100m", during the peak of the rut this 100m radius captured by this constraint could potentially include a number of harems. Further, females which associate are known to synchronise oestrus (Iason and Guinness 1985, but note this was not due to kinship *per se*), and the eleven day window we used as a temporal constraint may be too crude to capture this.

Regardless of the extent to which re-mating and intra-lineage polygyny can be explained by the temporal and spatial characteristics of the breeding system, these behaviours are associated with striking effects on the genetic structure of the population. Average relatedness was significantly higher in the observed pedigree than any simulation. Figure 5.1 demonstrates why this should be the case where intra-lineage polygyny exists: in this example, the relatedness of the two individuals is increased from 0.125 (aunt-half niece) to 0.375, because they also share a father, making them half sibs. Increased relatedness amongst groups has been implicated in social evolution, as it should promote cooperation between members of the same group (Hamilton 1964, Griffin and West 2003, Rossiter *et al.* 2005). Grazing behaviour in groups of red deer hinds is known to be affected by dominance and threats from one individual to another (Thouless 1990), but threats between matrilineal relatives are rarer and less intense than those to other hinds (Clutton-Brock *et al.* 1982). Females could therefore potentially be selected to mate with the same

male as relatives in order to increase the relatedness of their offspring, and so reduce the threat from female aggression to their offspring grazing within the matrilineal group.

However, as shown in figure 5.7, the mating behaviours observed in this study can also result in increased risk of inbreeding within matrilines. We found that average inbreeding coefficients of individuals were greater in the observed pedigree than under any of the random mating scenarios simulated (see figure 5.6). This increase was not driven by an increase in close inbreeding events, a similar level of which are predicted in all but the random mating scenario, but instead it seems likely re-mating and intra-lineage polygyny have contributed to increased numbers of 'deep' inbreeding events, such as that in figure 5.7. Theoretical studies indicate that female philopatry and consequent intra-lineage polygyny should only result in increased inbreeding within groups if there is a non random distribution of males with respect to relatedness (Storz 1999, see also Foerster *et al.* 2006). Indeed, although intra-lineage polygyny has also been found in horseshoe bats (Rossiter *et al.* 2005), in that case it was not associated with an increase in inbreeding events. Male site fidelity is likely to be important in generating close inbreeding events resulting from intra-lineage polygyny, such as father-daughter matings. However, deeper inbreeding events may result from male relatives rutting in the same area, particularly males rutting in the same place as their father, *i.e.* within their own natal groups. We have indeed found significant genetic structuring in the male population, indicating that male relatives are likely to be rutting in similar locations. In these cases, the magnitude of the inbreeding coefficient then has the potential to be inflated by intra-lineage events, as in figure 5.7: a relatively distant inbreeding event (aunt-half-nephew) is augmented by an instance of intra-lineage polygyny higher up the pedigree. In general therefore, it appears a non-random distribution of males with respect to relatedness, combined with the mating behaviours we have described, results in an increase in the risk of inbreeding over that which would be expected. In many lekking species, males have been shown to exhibit a non-random choice of mating sites with respect to relatedness (Piertney *et al.* 1999, Petrie *et al.* 1999, Shorey *et al.* 2000, Hoglund and Shorey 2003), and in grey seals, Pomeroy *et al.* (2001) found evidence males returned to their natal sites to breed, In lekking species, inclusive fitness benefits are generally implicated in such behaviour, as females may be preferentially attracted to larger leks (Shorey *et al.* 2000). Potentially, in the red deer system, dominant males may be more tolerant of subordinate males near their harem if they are related. In addition, given the short tenure of breeding males, relatives are unlikely to overlap in time as prime-aged individuals and so direct competition is rare.

Together, these factors could explain why males return to their natal areas to breed despite dispersing from their natal locations as young males.

Inbreeding is often associated with substantial fitness costs (Keller and Waller 2002). Why therefore is it tolerated in this population? Although many studies have documented fitness costs of inbreeding (reviewed in Keller and Waller 2002), several reviews have argued that inbreeding should be tolerated where the costs of inbreeding are not greater than the costs of inbreeding avoidance, such as costs of dispersal, loss of breeding opportunities, or costs of outbreeding; and that such conditions can be realistic (Kokko and Ots 2006, Waser *et al.* 1986, Bateson 1983). In particular, much theoretical attention has been paid to the idea that inbreeding tolerance can be favoured by inclusive fitness benefits. For example by mating with her brother, a female gains not only direct reproductive success but also indirect reproductive success via her brother, increasing the proportion of her genes which are passed on to the next generation (Parker 1979, Smith 1979, Waser *et al.* 1986, Kokko and Ots 2006). Inbreeding tolerance is only likely to evolve under such conditions if i) the cost of incestuous matings on offspring viability does not outweigh the inclusive fitness benefits of doing so and ii) the male does not lose other breeding opportunities by mating with his kin, which may be true for the red deer system in which male reproductive success is likely to be mostly limited by ability to gain access to females, rather than time or other ecological constraints (Smith 1979, Waser *et al.* 1986, Kokko and Ots 2006). It is worth considering that if mate copying is occurring in this population, a female mating with her male relative may result in other females copying her, increasing further the male's reproductive success and so the inclusive fitness benefits to the female. Further, the balance between costs of inbreeding and costs of inbreeding avoidance may be further tipped towards inbreeding tolerance by the potential benefits of intra-lineage polygyny in terms of cooperation between female relatives grazing (see above). Finally, the increase in inbreeding events which we have observed from that expected occurred due to an increase in distant inbreeding events, rather than those between close relatives: therefore the costs of inbreeding are reduced. No evidence for inbreeding avoidance has been found in a number of other polygynous systems (bighorn sheep: Rioux-Paquette *et al.* 2010; reindeer: Holand *et al.* 2006; great reed warbler: Hansson *et al.* 2006). In general it seems the expectation that animals should always avoid inbreeding requires further thought, and more work remains to be done to understand the evolution of inbreeding tolerance or avoidance in such systems.

That this study is conducted on an island population potentially increases the likelihood of the phenomena we have observed: for example, small populations restrict the opportunities for mating and therefore increase inbreeding risk (Keller and Waller 2002). However, comparison with mainland populations suggests these phenomena may be more widespread. An investigation of the dispersal of male and female red deer on the Scottish mainland concluded that whilst dispersal was predominantly male-biased, patterns of relatedness over geographical distances were similar for males and females (Pérez-Espona 2008). This study contrasted with the findings of previous work on the Rum population, which showed no spatial genetic structuring of the male population outside of the rut (Nussey *et al.* 2005b). However, interestingly, males in the mainland study were sampled during the hunting season (1st July to 20th October), which partly overlaps with the rutting period; the period in which our results indicate spatial genetic structure amongst males in the Rum population.

Conclusions and areas of future research

Using molecular paternity analysis, we have revealed more re-mating between pairs and more intra-lineage polygyny in a population of wild red deer than expected. Combined with hitherto un-quantified genetic spatial structuring of the rutting male population, these mating behaviours are associated with increased relatedness of individuals in the population, but also an increase in inbreeding events. Such behaviours are extremely rarely documented in wild polygynous mammals, in part because of the challenge of collecting sufficient data across generations to identify them; yet they are key tests of theoretical concepts of population genetics. In general, the combined use of molecular paternity analysis and simulated pedigrees based on potential mating scenarios has revealed further the hidden complexity of this polygynous mating system, and raised many interesting questions for future research: the role of female choice or mate copying, the implications for social evolution and the extent to which inbreeding should be tolerated or avoided in such systems. Identifying, and understanding, such phenomena in wild populations is also critical to wider areas of research. For example, estimates of quantitative genetic parameters, such as trait heritabilities, may be confounded by inflated relatedness amongst closely spatially associated individuals.

Chapter 6:
Accounting for shared environment effects on quantitative genetic parameters: spatial autocorrelation and the 'double-matrix' approach reduce estimates of heritability

6.1 Summary

Social structure and limited dispersal are ubiquitous in wild vertebrates. As a result, relatives often share environments as well as genes, such that environmental and genetic sources of similarity between individuals are potentially confounded. This is likely to result in upward bias of estimates of the contribution of additive genetic variance to phenotypic similarities between individuals, and hence estimates of trait heritability. In this study, we demonstrate two approaches to estimate and account for these shared environment effects in animal models, linear mixed effects models in which quantitative genetic parameters are estimated using pedigree data. In the first method, we fit a 1st order separable auto-regressive process in two dimensions to account for spatial autocorrelation in trait values. Secondly, we develop a novel multi-matrix technique, in which the genetic and shared environment components of variance are estimated simultaneously by fitting a second matrix to the animal model in addition to the pedigree. This second matrix, termed the 'S matrix', denotes the extent to which pairs of individuals overlap in their home ranges. Using these techniques, we demonstrate that shared environment effects greatly upwardly bias estimates of heritability. In spatial traits, (home range size) inclusion of the home range overlap information substantially reduced estimates of heritability, by up to 98%. Further, estimates of heritability of lifetime breeding success were reduced by 70%, and of birth weight by 10%. Our findings therefore show significant bias exists in estimates of heritability from animal models when shared environment effects are not accounted for, and demonstrate novel methods for the resolution of this issue in wild animal quantitative genetics.

6.2 Introduction

The variation observed in nature between individuals can be explained by genetic effects, environmental effects, or a combination of both. Additive genetic variance (V_A) and heritability (h^2, the ratio of genetic to phenotypic variance) can be estimated from phenotypic similarities between relatives (Lynch and Walsh 1998). These evolutionary parameters are fundamental for estimating response to selection and so the potential for a population to evolve (Lande 1982, Houle 1992). As a result, a great deal of effort has been directed at estimating heritability in wild populations (Réale and Fiesta-Bianchet 2000, Kruuk 2004, Roff and Rausher 2007, Kruuk *et al.* 2008). Statistical techniques used to estimate V_A and h^2 range from parent-offspring regression to "animal models", mixed-effects models in which the phenotypic covariance of all pairs of relatives in the population is assessed using multi-generational pedigree data (Lynch and Walsh 1998, Kruuk 2004). The use of animal models in wild animal quantitative genetics has led to considerable recent advances in our understanding of the evolutionary genetics of natural systems (Kruuk 2004, Ellegren & Sheldon 2008, Pemberton 2010). However, in contrast to lab studies which measure V_A under largely homogeneous conditions, natural populations are characterised by high levels of environmental heterogeneity, and this can potentially bias estimates of quantitative genetic parameters (Kruuk and Hadfield 2007). To date, efforts to examine and account for such bias have been largely limited to early-life effects (such as maternal or nest effects, *e.g.* Kruuk *et al.* 2000, Charmantier *et al.* 2004, MacColl and Hatchwell 2003, Wilson *et al.* 2005). In this study, we examine the contribution of spatial autocorrelation and lifelong patterns of home range overlap to phenotypic variation and their potential to bias estimates of V_A and h^2 in a wild red deer population.

Social structure and limited dispersal can generate long-lasting environmental associations between relatives, so that the potential for relatives to share environments as well as genes is often high. The causes and consequences of this are an interesting focus of research in themselves. For example, studies have addressed why relatives do not completely disperse, and whether they benefit from associating in the same environment, particularly through kin selection (Höglund *et al.* 1999, Rossiter *et al.* 2005, Storz 1999). Genetic structure of populations is of particular interest for studies of mate choice via inbreeding avoidance because it affects both the likelihood and cost-benefit ratio of inbreeding (*e.g.* Foerster *et al.* 2006, Reid *et al.* 2010). Finally, genetic structure and social barriers to

dispersal can affect demography (Lambin and Yoccoz 2002) and result in spatial heterogeneity of fitness across a population (Conradt *et al.* 1999).

However, social structures which result in relatives sharing environments are also likely be a source of bias in estimates of V_A and h^2. This is because environmental and genetic sources of similarity between individuals are potentially confounded where relatives associate in the same environment (Coltman *et al.* 2003). It has been argued that such sources of environmental bias may explain why phenotypic changes are rarely correlated with those predicted by selection (Merilä *et al.* 2001a, Van der Jeugd and McCleery 2002). Disentangling the extent of this bias will be important in determining the relative roles of plasticity and genetic change in population response to environmental change, such as that caused by anthropogenic climate change (Gienapp 2008). Furthermore accounting for this bias is critical in accurately distinguishing between genetic and environmental effects in studies of genotype by environment interactions, because the relative magnitude of environmental effects may be greater under unfavourable conditions (Hoffman and Merilä 1999, Charmantier and Garant 2005).

Spatial autocorrelation (SAC), the dependence of a given variable's value on the values of the same variable measured at nearby locations (Cliff and Ord 1973, Fortin and Dale 2005), has long been recognised as a source of bias in quantitative genetic analyses of agriculture and forestry studies (e.g. Cullis and Gleeson 1989, Cullis and Gleeson 1991, Burgueño *et al.* 2000, Costa e Silva *et al.* 2001). It has also been widely considered more generally in ecology, both as a source of bias but also in identification of relevant and interesting spatial processes (Legendre 1993, Kissling and Carl 2008, Fortin and Dale 2009). However, its importance is only recently being recognised in other fields, such as behavioural ecology and wild animal quantitative genetics (*e.g.* Durães *et al.* 2007, De Solla *et al.* 2001, Giesselmann *et al.* 2008, Van der Jeugd and McCleery 2002). Where high values of a variable are associated with high values in space, SAC is positive; where high values are associated with low values, SAC is negative. SAC may arise because of intrinsic properties of the variable itself, usually through inter-individual competition. For example, in territorial species, small territories are intrinsically likely to be near other small territories because of the density of individuals competing. Alternatively, SAC may arise through dependency of the variable on other spatially autocorrelated attributes, such as the dependence of clutch size on food availability (Valcu and Kempenaers 2010). Importantly, ignoring SAC ignores a form of pseudoreplication, as it violates the

assumption of linear models (and other statistical tests) that residuals should not be correlated. Therefore models ignoring SAC potentially employ inflated sample sizes, leading to erroneous findings (Hulbert 1984, Haining 2004, Legendre 1993). Understanding SAC can also be useful more broadly, providing insight into the scale over which resources are heterogeneous, or the scale on which individuals interact (Valcu and Kempenaers 2010).

In quantitative genetic analyses of agricultural and forestry trials, SAC can be accounted for to some extent by experimental design and appropriate fitting of block effects. However, particularly in forestry trials, substantial heterogeneity may exist in the sites used which can be further modelled by the inclusion of SAC terms (Dutkowski *et al.* 2002). Using simulations, Magnussen (1993) showed that estimates of variance components were upwardly biased where positive SAC was not accounted for, although other forestry studies have found that accounting for SAC can have varying effects on estimates of additive genetic variation (Costa e Silva *et al.* 2001, Dutkowski *et al.* 2002). In contrast to forestry, in studies of wild animals, SAC in estimates of quantitative genetic parameters has received little attention, with the exception of one study by Van der Jeugd and McCleery (2002). Van der Jeugd and McCleery (2002) examined the effects of SAC in laying date of great tits on estimates of heritability from parent-offspring regression. In parent-offspring regressions, heritability estimates are generally inflated when parents and offspring share the same environment (Falconer 1989). Van der Jeugd and McCleery accounted for this by estimating both the apparent genetic heritability of laying date and the 'heritability' which would be observed in this trait as a result of SAC. They found that more than 60% of the estimated "heritability" of laying date was in fact due to SAC, and that the true heritability of the trait was substantially lower when SAC was corrected for. The extent to which heritabilities of traits are inflated by SAC is likely to be dependent upon the trait considered, due to both the extent to which the trait is spatially autocorrelated, and the relative importance of genetic and environmental effects on the trait. For example, Van der Jeugd and McCleery (2002) found that clutch size was also spatially autocorrelated, although less so than laying date, but that SAC did not explain the high estimate of heritability of clutch size. Relatively plastic traits which are strongly affected by environmental components are likely to be the more spatially autocorrelated (given a heterogeneous environment), as are traits which depend upon interactions between individuals (Van der Jeugd and McCleery 2002, Valcu and Kempenaers 2010).

Rather than explicitly modelling SAC, the inclusion of common environment effects in animal models is often advocated to deal with the problem of shared environments (Kruuk 2004, Kruuk and Hadfield 2007). This is achieved by fitting various effects that can increase the phenotypic covariance between relatives as additional random effects: maternal or paternal effects, nest or brood effects, cohort effects and so on. Doing so has demonstrated the importance of shared environments (*e.g.* Wilson *et al.* 2005, Merilä *et al.* 2001b, Kruuk and Hadfield 2007). For example, shared nest effects accounted for 43% of variance in helping behaviour in long tailed tits (MacColl and Hatchwell 2003). Using simulated data, Kruuk and Hadfield (2007) showed that considerable bias exists in estimates of heritability of parturition date in Soay sheep, antler size in red deer and birth weight in red deer if shared environmental effects are not accounted for in animal models. These biases were reduced when common environment or maternal effects were modelled. However, accounting for shared environment effects through inclusion of such parameters requires data on the shared environmental factors likely to be important, and the ability to fit these factors to the model without over-parameterization. Although fitting a common environment effect, such as nest box, in an animal model can account for the similarity between individuals due to sharing the nest environment, this will not account for other environmental effects which occur before or after leaving the nest, such as relatives wintering in similar locations. A comparison of the findings of Van der Jeugd and McCleery (2002) with those from an animal model suggest that heritability of laying date would be over-estimated by 30% using an animal model with a nest box effect versus a parent-offspring regression corrected for SAC (McCleery *unpublished data*, cited in Van der Jeugd and McCleery 2002). This demonstrates that including such crude environmental data in animal models is insufficient as a method of eliminating co-variation between relatives. However, to our knowledge, no study has yet attempted to fit spatial auto-correlation processes to animal models of traits of wild animal populations.

The present study

In this study, we attempt to account for environmental sources of bias in estimates of heritability due to environmental co-variation between relatives in a wild population of red deer. In red deer, females are strongly philopatric, with little dispersal from their natal sites, and the majority of females associate in loosely matrilineal groups (Clutton-Brock *et al.* 1982, Albon *et al.* 1992). There is therefore fine scale genetic structure within the

female population (Nussey *et al.* 2005b). Related females live in similar environments, and hence are likely to experience similar environmental effects.

The effect of matrilineal relatives sharing environments on estimates of heritability of offspring birth weight (as a trait of the offspring) was investigated by Kruuk and Hadfield (2007) by inclusion of a matrilineal term in an animal model of the trait. The authors showed that some variance in birth weight which could be attributed to an individual's matriline was incorporated into estimates of additive genetic variance when matriline was not fitted in the model, such that the heritability was inflated from 7.5% to 13.5%. This indicates that the model partly confounded genetic similarity between females and covariance between females due to living within the same matrilineal group. Further, when maternal genetic effects were fitted to animal models of birth weight that did not include a matriline term, some of the variance due to matriline was incorporated into the maternal genetic term. This indicates that the estimate of the maternal genetic effect was also confounded by the association of female relatives.

In this study, we use two methods to account for shared environment effects in animal models. We firstly fit a 1st order separable auto-regressive process in two dimensions (row and column, Gilmour 1997, Cullis and Gleeson 1991) to model the SAC in each trait. Secondly, we use a 'double-matrix' animal model, in which additive genetic effects are estimated from the phenotypic covariance between relatives identified in a genetic pedigree, but in which a shared environment component is estimated simultaneously from a second matrix. In this second matrix, the phenotypic covariance between individuals is related to the degree to which they share a spatial environment: specifically, this matrix, here termed the 'S matrix' to denote shared space use, defines the extent to which the area of females' home ranges overlap. Using these approaches, we attempt to separate phenotypic covariance due to shared space use from phenotypic covariance due to shared genes, and thus examine the bias in estimates of heritability due to environmental co-variance between relatives.

We use the SAC and double-matrix approaches to account for bias in estimates of heritability of four female traits: home range size during the rut, home range size during spring, birth weight as a trait of the mother, and lifetime breeding success. We chose heritability of home range size in which to investigate shared environment effects because home range size is likely to be spatially-autocorrelated due to its dependency on food

availability. Females are expected to trade off the range size needed to acquire sufficient food with the energy required to move across this range (McNab 1963); therefore home range size will be dependent upon the availability and quality of forage and so is expected to vary spatially over the study area. Furthermore, relatives are known to overlap in their home ranges (Moyes 2007). We therefore expect to find shared environment bias in estimates of heritability of home range size and so these traits are illustrative of the potential of the techniques we employ. Birth weight has received substantial attention in this study population, with respect to climate (Albon *et al.* 1983), quantitative trait loci (Slate *et al.* 2002), selection (Coulson *et al.* 2003), plasticity (Nussey *et al.* 2005a), heritability (Kruuk and Hadfield 2007), individual variation in response to changes in population density (Stopher *et al.* 2008), senescence (Nussey *et al.* 2009) and most recently, response to climate warming (Moyes *et al.* 2011). Understanding the relative role of genetic and environmental variation in this trait is crucial to interpreting the findings of these studies and for future investigation of how the trait may evolve in response to climate warming, yet to date the effect of spatial autocorrelation and home range overlap on this trait have not been investigated. Finally, lifetime breeding success is a trait of crucial evolutionary importance as a measure of individual fitness across many taxa (Coltman *et al.* 1999, Merilä and Sheldon 2000, Rodríguez-Muñoz *et al.* 2010). In particular, many authors have been interested in whether fitness traits have low heritabilities, and whether this is due to the magnitude of environmental variance in such traits (Merilä and Sheldon 2000). Distinguishing genetic from environmental causes of similarities between relatives in lifetime breeding success is critical to answering this question.

6.3 Methods

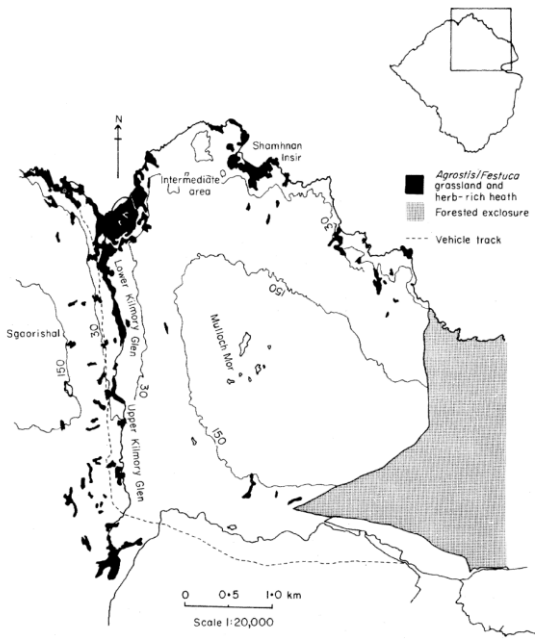
Study population and data collection

The data in this study is taken from a wild population of red deer, *Cervus elaphus*, on the North Block of the Isle of Rum, Scotland, which has been intensively monitored since 1971. All individuals in the population can be recognised by artificial markings or natural idiosyncrasies (Clutton-Brock *et al.* 1982). The study population was released from a culling regime in 1973, and the population size then rose steadily towards carrying capacity in the mid 1980s, with the current population fluctuating around approximately 200 adult females (Coulson *et al.* 2004). Females in this population associate in loosely matrilineal groups (although this is known to have declined somewhat over the early part

of the study, 1974-1983, Albon *et al.* 1992). In contrast to females, young males disperse from their natal groups at around two years of age (Clutton-Brock *et al.* 1982). Males born to the study population often return to the study area to rut, but outwith the rut essentially all adult males live outside the study area for the majority of the year. Little spatial information is therefore available for males.

The study area is approximately 13km^2, comprising a gently sloping hill (Mulloch Mor) and the surrounding glens, with the majority (more than 70%) of the area lying below 120m (Clutton-Brock *et al.* 1982). The north boundary of the study area follows 3.5km of coastline from Kilmory Bay to another bay, Shamhan Insir to the East (see figure 6.1). Females spend most of their time feeding along this coastal strip and on the bottom of the 4km section of Kilmory river that runs down Kilmory Glen, draining into the bay (Clutton-Brock *et al.* 1982, Coulson *et al.* 2004, McLoughlin *et al.* 2006). Five main types of vegetation have been classified in the study area: *Agrostis/Festuca* grassland, *Juncus* dominated marshland, *Molinia* dominated flush, *Calluna* dominated heath and heather moorland and small patches of *Eriophorum* dominated bog (Clutton-Brock *et al.* 1982, McLoughlin *et al.* 2006). There is considerable heterogeneity of these vegetation types across the study area; in particular use of *Agrostis/Festuca* has been associated with positive effects on lifetime reproductive success (McLoughlin *et al.* 2006).

Figure 6.1: the study area, showing distribution of *Agrostis/Festuca* grassland. From Guinness *et al.* 1978.



During the calving season, detailed observations are taken of heavily pregnant females to identify when and where calves are born. This allows the majority (64% over the whole study period) of individuals born into the population to be caught shortly after birth, when they are sexed, weighed and sampled for genetic paternity analysis (see below). Capture weight is adjusted for the time since birth to give a birth weight for each individual in kilograms (Birth weight= capture weight-0.01539 x age at capture (hours), see Clutton-Brock *et al.* 1982 for more details). Survival of calves is monitored by censusing (see below) and searching for corpses throughout the year. In any year, females can be classified into five reproductive status categories dependent upon whether they gave birth the previous year, and if so, whether the calf survived: milk (calved, and calf survived to at least May 1st the year after birth), winter yeld (calved, but calf died in the winter after birth, between 1st October and 1st May), summer yeld (calved, and calf died before 1st October in the year of birth), true yeld (the female did not calve the previous year) and naïve (the female had never previously calved). These categories are associated with breeding performance in the current year (Coulson *et al.* 2003).

Locations of individuals during spring were taken from censuses conducted five times a month during the period of January to May. During a census, a fixed route is walked through the study area and the identity of all individuals seen is recorded and their grid reference noted to the nearest 100m. Although censuses are undertaken throughout the year, the data used here was restricted to the January to May period because at other times individual location may be temporarily affected by calving or mating behaviours. During the rut (15th September to 15th November), censuses are undertaken daily, again recording identity and location of individuals to the nearest 100m. Females are considered to range mainly within one of five regions in the study area from their mean lifetime locations: Kilmory, Shamhan Insir, Intermediate area, Mid glen or South glen (Moyes 2007). Using this classification, we calculate local population size in this study for each region annually as the number of adult females whose mean location falls within each region.

Analysis

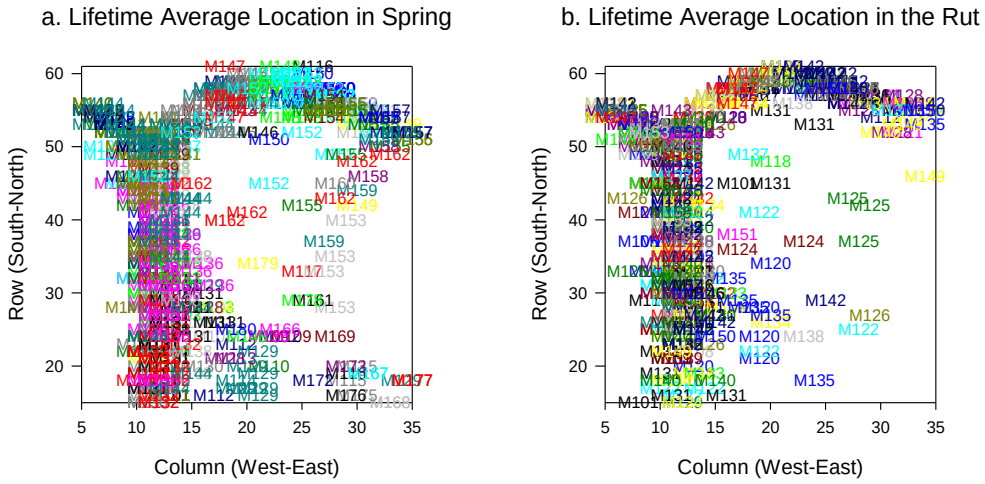
Spatial analysis

1. Spatial Grid for SAC analysis

For the purposes of spatial analyses, the locations in which individuals were recorded were transformed on to a grid, so that the most south-westerly location recorded (135100,

798500) became (0, 0) and each step along the grid in either direction represented a shift in location by 100m. Positions on the grid were then represented by a grid reference (column, row). Average lifetime locations of individuals on this grid are plotted in figure 6.2a (average location during January to May) and 6.2b (average location during the rut), grouped by the matriline to which the individual belonged. These lifetime average locations were then used to account for spatial autocorrelation in animal models (see below).

Figure 6.2a and 6.2b: distribution of average female lifetime locations in a) spring and b) the rut, coloured and labelled by the matriline to which the female belonged.



2. Home Ranges

Because censuses record the grid references of individuals to the nearest 100m, many fixes have exactly the same grid reference. This can cause problems in the calculation of home range sizes and overlap using kernel methods (Tufto *et al.* 1996). To address this, we 'jittered' locations used for home range estimation by adding a random number between -20 and 20 to the X and Y coordinates for each grid reference (Moyes 2007).

Home range size

Home range sizes were estimated for each female annually using locations recorded within a) spring (January to May) and b) the rut. Where less than ten locations were recorded for an individual during a particular season, the data was excluded for that female. Borger *et al.* (2006) showed that this number was sufficient for accurate home range estimation using techniques similar to those used here; further, we investigated the

number of fixes used to estimate a home range as a fixed effect in subsequent analyses to account for any bias in home range size which may result.

Home ranges were estimated using kernel density estimation methods (Borger *et al*. 2006; Worton 1987; Worton 1989). This type of home range estimation has revolutionised the concept of an animal's home range, as it takes into account the utilization distribution of the animal, that is, the probability distribution defining the animal's use of space (Fieberg and Kochanny 2005, Van Winkle 1975, Kenward *et al.* 2001, Worton 1989). It has been recommended that multiple smoothing parameters are used and the results compared (Borger *et al.* 2006). We attempted to model home range sizes using both the reference bandwidth, h_{ref}, method and the least squares cross validation method; however the latter method performed poorly and would not converge, a finding which has been predicted by other studies for home ranges with relatively small numbers of fixes (Seaman and Powell 1996). We therefore used the h_{ref} method. We estimated home ranges using the package 'adehabitat' (version 1.8.3, Calenge 2006) in R version 2.8.1 (R Development Core Team 2008).

We estimated home ranges for each individual in each season at a very wide range of isopleths (between 20 and 95% at 5% intervals), where isopleths indicate the probability of finding an individual within the calculated home range. If home ranges are used randomly, then a positive linear correlation exists between isopleth and home range size; however, if animals use part of the home range more intensely then a plot of home range size against isopleth will be curved beneath the line of random use. At the point at which the difference between random use and actual use is greatest, the isopleth represents the 'core home range' of the animal (Clutton-Brock *et al.* 1982, Powell 2000, Moyes 2007). It has been suggested that SAC is particularly likely to be an issue in core home ranges (Borger *et al.* 2006). In our analysis, the core home range was found to be that with a 70% isopleth for both spring and rut home ranges. The size of the 70% isopleth home range in hectares is therefore the trait we refer to as 'home range size' in our analysis.

Home Range Overlap

We estimated the extent of space-sharing among individuals. To do this, we used home ranges calculated as above, but using all locations recorded over an individual's lifetime rather than annual locations. Although home range overlap can be calculated as a simple proportion of an animal *i*'s home range that is overlapped by an animal *j*'s home range

(Kernohan *et al.* 2001), incorporating information about an animal's utilization distribution (UD) can result in more informative measures of home range overlap. Not doing so can result in a large overlap estimate even though the probability of finding two individuals in the same place is small (Fieberg and Kochanny 2005). Fieberg and Kochanny (2005) recommend the use of either the UD overlap index (UDOI) or Bhattacharyya's affinity (BA; Bhattacharyya 1943) as measures for quantifying home range overlap on this basis. UDOI is arguably the most appropriate measure of shared space use, because it can take into account the degree to which utilization distributions are concentrated in space, whereas BA is more appropriate to quantify the overall similarity between UDs. However, BA has the advantage that it ranges from zero to 1, equalling one when UDs are uniformly distributed with 100% overlap. Here, we used BA and we calculated home range overlap at a 100% isopleth rather than a (core) 70% isopleth as was used for home range size. By using BA and calculating home range overlap at 100%, individuals have an overlap of 1 with themselves. Scaling from 0-1 in this way makes scaling of the overlap term comparable to that of relatedness between two individuals, which would not be the case if using the UDOI method. This is critical when comparing the variance in a trait explained by the relatedness and spatial matrix because the variance explained by each matrix must be on the same scale. We calculated a home range overlap matrix for all individuals in the genetic pedigree; where no home range information was available for an individual, it was assigned a home range overlap index of 1 with itself (diagonals set to 1), and was assumed to have an overlap of zero with all other individuals (missing off-diagonals assumed to be zero). Compared to 4051 individuals (but only 1384 females) in the pedigree, home range information only existed for 948 females in spring and 766 females in the rut. Males necessarily had no home range information, as spatial data for males is available to a far lesser degree. However, in comparison, the pedigree also contains missing information, as 691 individuals have no known mother or father, and further this lack of information in the spatial matrix is only likely to make our estimates of the variance in a trait explained by home range overlap more conservative.

Pedigree reconstruction

All mothers are known through association with their calves, whilst genetic paternity analysis was used to assign fathers. As discussed above, the majority of individuals are caught at birth and samples taken for paternity analysis. Genetic sampling for individuals not caught at birth occurs from cast antlers, chemical immobilization or post-mortem. Prior to 1991, individuals were genotyped at up to 8 highly variable microsatellites; since

then individuals have been genotyped at up to 15 microsatellites. Detailed methods of pedigree construction are given in Walling *et al.* (2010). Two programs were used for paternity assignment: MasterBayes (Hadfield *et al.* 2006) and COLONY2 (Wang and Santure 2009). All assignments were made at greater than 80% individual confidence. Preference was given to paternity assignments made by MasterBayes, with COLONY2 assignments accepted where MasterBayes could not assign a father with greater than 80% confidence.

Animal models

Animal models were conducted in ASReml3 (Gilmour *et al.* 2009). Significance of random effects was assessed using likelihood ratio tests, with twice the difference between log likelihoods assumed to be χ^2 distributed with degrees of freedom equal to the difference between the models in the number of parameters estimated.

We considered the effects of spatial heterogeneity on estimates of variance components in four traits separately: annual rut home range size (RHR), annual spring home range size (SHR), offspring birth weight as a trait of the mother (BW), and lifetime breeding success (the number of offspring a female produced in her lifetime, LBS). It was necessary to log-transform RHR, SHR and LBS in order that the distribution of the residuals had a closer approximation to normality.

We attempted to decompose the variance in these traits into permanent environment effects, additive genetic variance, year (year of birth for LBS) and maternal effects. We then investigated (i) the effect of including SAC in the model on estimates of variance components of the four traits and (ii) how home range overlap affected our estimates of the variance components of these traits, by fitting a matrix of home range overlap (the 'S' matrix) to the animal model.

Fixed effects were fitted to these models to account for variation due to female age or reproductive status where appropriate. Fixed effects related to spatial processes were also fitted in some models, including region of the study area or local population size. These potentially account for some of the spatial heterogeneity in these traits (for example, fitting region to birth weight was confounded with SAC, see results). However, trends in the variables which result from large scale spatial heterogeneity ('global trends') can inflate SAC, particularly if they are aligned with column or row effects (Dutkowski *et al.* 2002). In this population this is likely because, for example, local population density

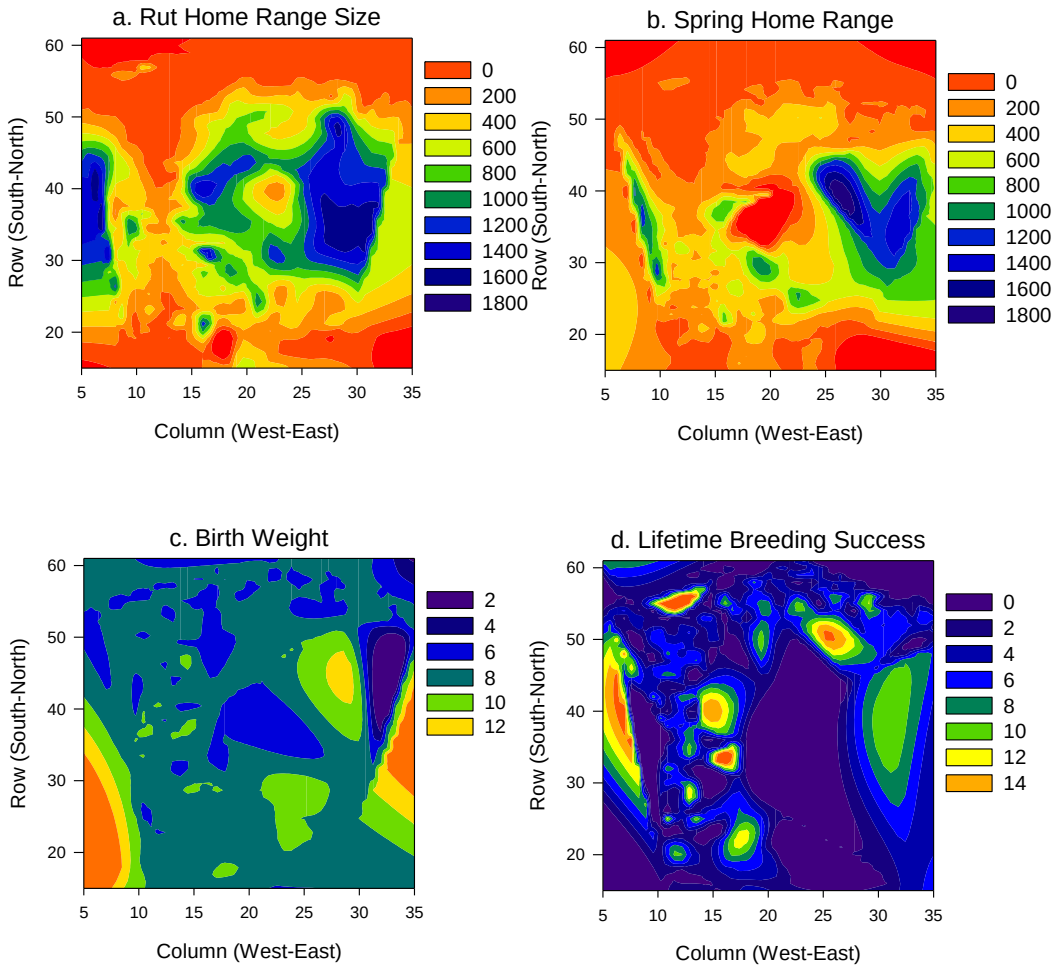
broadly increases from South to North (Coulson *et al.* 2004). The spatial distribution of the four traits considered is shown in figures 6.3a-d, for consideration of global trends and other spatial heterogeneity in the traits. It has been argued that although fitting such trends is unlikely to change estimates of quantitative genetic parameters, where it can be done easily it can aid our understanding of the nature of variation, and improve the likelihood of achieving stationarity in the SAC processes (Dutkowski *et al.* 2002). The following significant fixed effects were identified from Moyes (2007), Coulson *et al.* (2003) and preliminary analysis (data not shown) and were fitted in all models except were explicitly stated:

Fixed effects fitted were:

RHR: age, region, local population size, count of fixes used to calculate home range size.
SHR: age, and its quadratic term, local population size, region, reproductive status.
BW: mother's age and its quadratic term, the mother's region, the mother's reproductive status, and sex of offspring.
LBS: region.

Note, count of locations used to calculate home range size did not have a significant effect on SHR and so was not included.

Figure 6.3: spatial distributions of **a)** rut home range size, **b)** spring home range size, **c)** birth weight and **d)** lifetime breeding success. Where data is not available for a grid square, the expected value for that square is interpolated from those around it (using SigmaPlot, Systat software 2008).



1. Spatial Autocorrelation

We investigated whether SAC affected our estimates of variance components. We fit animal models sequentially, fitting first a permanent environment effect, then an additive genetic term, a year effect and finally a maternal effect for RHR, SHR and BW, and an additive genetic term, year of birth (cohort effect) and then a maternal effect for LBS. To each step of these models, we fitted a 1st order separable autoregressive process (AR1xAR1, Gilmour 1997). In AR1, the autocorrelation is a power function of the distance apart; in two dimensions separable autoregressive processes for columns and rows are modelled such that $r(X_{i,j}, X_{k,l}) = \rho row^{|i-k|}\rho col^{|j-l|}$ for individuals with row (i,j) and column (k,l) coordinates (Cullis and Gleeson 1991). We modelled such processes as

additional variance structures through the G matrix, as this does not require a rectangular distribution of individuals (Apiolaza 2006). Doing so estimates both the correlation parameter and the variance in the trait explained by the spatial term. We fitted i) a column process, ii) a row process and iii) column and row processes. Average lifetime locations (column, row) were taken from rut censuses for fitting to models of rut home range size and from spring censuses for fitting to models of the other traits. We then examined the significance of the change in log likelihood to test whether fitting SAC processes improved the model fit.

2. Home Range Overlap

We investigated whether the degree to which individuals overlap in home ranges explained a significant amount of phenotypic covariance in the traits considered. Again, we fitted animal models sequentially, fitting permanent environment effects (not for LBS), an additive genetic term, a year effect (birth year for LBS, *i.e.* a cohort effect) and then a maternal effect. To each step of these models, we also fitted the second matrix of home range overlap between individuals, and compared the models using chi squared tests on the difference in log likelihoods. Lifetime rut home range overlap was fitted to models of rut home range size, lifetime spring home range overlap was fitted to models of the other three traits.

There was significant similarity between the A matrix (the matrix of relatedness between individuals) and both the rut home range overlap matrix (Mantel test of similarity with 10000 permutations, $Z=2487.955$, $p<0.0001$) and the spring home range overlap matrix (Mantel test of similarity with 10000 permutations, $Z=3907.416$, $p<0.0001$), confirming that relatives overlap in their home ranges.

6.3 Results

We would like to note a caveat which applies to the results of the spatial autocorrelation analyses. Whilst adding SAC processes to animal models of the traits considered has revealed interesting environmental sources of variance (see below), not all the models we considered produced credible results. In particular, some estimates of the variance explained by spatial processes were extremely large. The credibility of these can be checked by summing the variance components to give the total phenotypic variance estimated by each model (see table 6.1); whilst some minor changes in the total variance explained are not necessarily uncommon (I. White, *pers. comm.*) changes of the order of

magnitude of the total variance are more alarming and suggest the model has produced a poor estimate of the variance components. This occurred particularly when the estimates of the SAC were bound at 1 (*i.e.* could not be estimated). Therefore, here we discount models in which the sum of the variance components is more than an order of magnitude greater than in other models of the same trait.

1. Rut home range size (RHR)

SAC

There was a significant improvement on fitting SAC to models of RHR (see table 6.1a). Fitting row, or column and row processes to the model resulted in maternal effects being no longer significant, dropping from explaining 15.75±3.13% of the variance to 1.97±0.85% in the model with column and row. This suggests the apparent maternal effect is actually caused by environmental covariance between maternal relatives living in similar locations and therefore experiencing similar environmental effects on rut home range size. Given maternal effects were still significant when only column processes were included (see table 6.1a), this similarity must occur primarily along the North-South continuum.

The most parsimonious model of rut home range included a permanent environment effect (although this was bound at zero, *i.e.* it could not be estimated), an additive genetic term, a year effect, and spatial column and row autocorrelation processes (see table 6.1a). In this model, the heritability of the trait was reduced substantially (by 89%) from 48.75±2.17% in a model with no SAC processes to 5.47±1.71 in the final model (see figure 6.4a). The proportion of variance explained by the spatial term was large (72.18±8.3%). Compared to the model with no SAC, this variance came mainly from a reduction in additive genetic variance, but also from the year effect and the residual term. Therefore, most of the covariance in rut home range size due to similarity between individuals living in similar locations would have been ascribed to additive genetic variance had SAC not been considered.

Home range overlap

Individuals whose home ranges overlapped were likely to have rut home ranges of similar size: there was significant variance due to home range overlap effect in rut home range size (see table 6.2a). Similarly to the inclusion of SAC, when home range overlap was

fitted, maternal effects on this trait were no longer significant (see table 6.2a). Therefore again not including shared environment resulted in erroneously assigning similarity between individuals to a maternal effect. Adding the overlap term to a model of permanent environment, additive genetic variance and year explained a substantial proportion of the variance in this trait (68%), which resulted in a large reduction in the proportion of variance explained by the additive genetic term, from 48.75±2.17% to 0.76±3.20%, as well as the year and residual terms (see table 6.2a and figure 6.4a).

2. Spring Home Range

SAC

Again, fitting SAC significantly improved models of spring home range size (see table 6.1b). We found no evidence for maternal effects in this trait. Adding column processes to a model including a permanent environment effect (although this was again bound at zero), an additive genetic effect and a year effect reduced the heritability of spring home range size from 48.3±1.9% (no spatial processes) to 24.5±3.2%, again indicating that much of the variance that appears to be explained by genetic similarity between individuals was in fact due to covariance between individuals in similar environments (see figure 6.4b). As in RHR, this spatial variance came from not just the additive genetic term but also the year and residual variance (see figure 6.4b). The model with the highest likelihood contained both row and column processes but was discounted due to inflation of the variance components (table 6.1b, see above).

Home Range Overlap

There was also a significant home range overlap effect on spring home range size (see table 6.2b). Again, there were no significant maternal effects. Adding the overlap term to a model containing a permanent environment effect, an additive genetic effect and year explained 68.68±3.92% of the variance in SHR, and inclusion of this term reduced the estimate of heritability enormously from 48.31±1.89% to 0.37±0.21% (see figure 6.4b); indeed adding V_a to a model containing a permanent environment effect and overlap term was only just significant (p=0.0409).

3. Birth Weight

SAC

Although we had intended to fit region as a fixed effect in models of birth weight, we found the model contained singularities when SAC was included and region was fitted as a fixed effect. This suggests that the two effects are heavily confounded. We therefore present models without region fitted as fixed effects. These can be compared to models of BW with region fitted by examination of table 6.2c. Overall, the models of birth weight with SAC were generally quite unstable (for example, see the large standard errors of spatial variance components and the frequency with which spatial processes were bound at 1, table 6.1c); although we present them for discussion the results should be treated with caution. Note also that one model (which contained permanent environment, additive genetic, year and column processes) is not reported as the model did not converge.

With region not included as a fixed effect, fitting SAC significantly improved models of offspring birth weight (see table 6.1c). Adding column and row processes to a model of birth weight including a permanent environment effect, additive genetic effect, year and maternal effect reduced the estimate of heritability, from 35.61±5.13% to 26.28±6.98% (see figure 6.4c). Spatial processes contributed 19.54±22.44% of the variance in this model. The extremely large standard error on this is a probably a result of spatial processes in one direction, in this case column, being bound at 1; the sum of the variance components was not particularly inflated in this model but the variance components described should perhaps be treated with caution.

Home Range Overlap

There was a significant home range overlap effect on birth weight (see table 6.2c). P values in this analysis are considered significant as they are below 0.05, although a Bonferroni correction for the four tests of adding the overlap term would put the critical significance at 0.0125. We choose to accept the less conservative P value because adding home range overlap was significant in each of the four models, and therefore our findings are unlikely to constitute a Type 1 error.

In a model containing a permanent environment effect, an additive genetic effect, year, a maternal effect, and the home range overlap term, home range overlap explained a small

percent of the variance (5.92±4.76%); and there was a small reduction in heritability (from 28.21±5.66% to 25.60±5.54%) when overlap was included (see figure 6.4c).

4. Lifetime breeding success

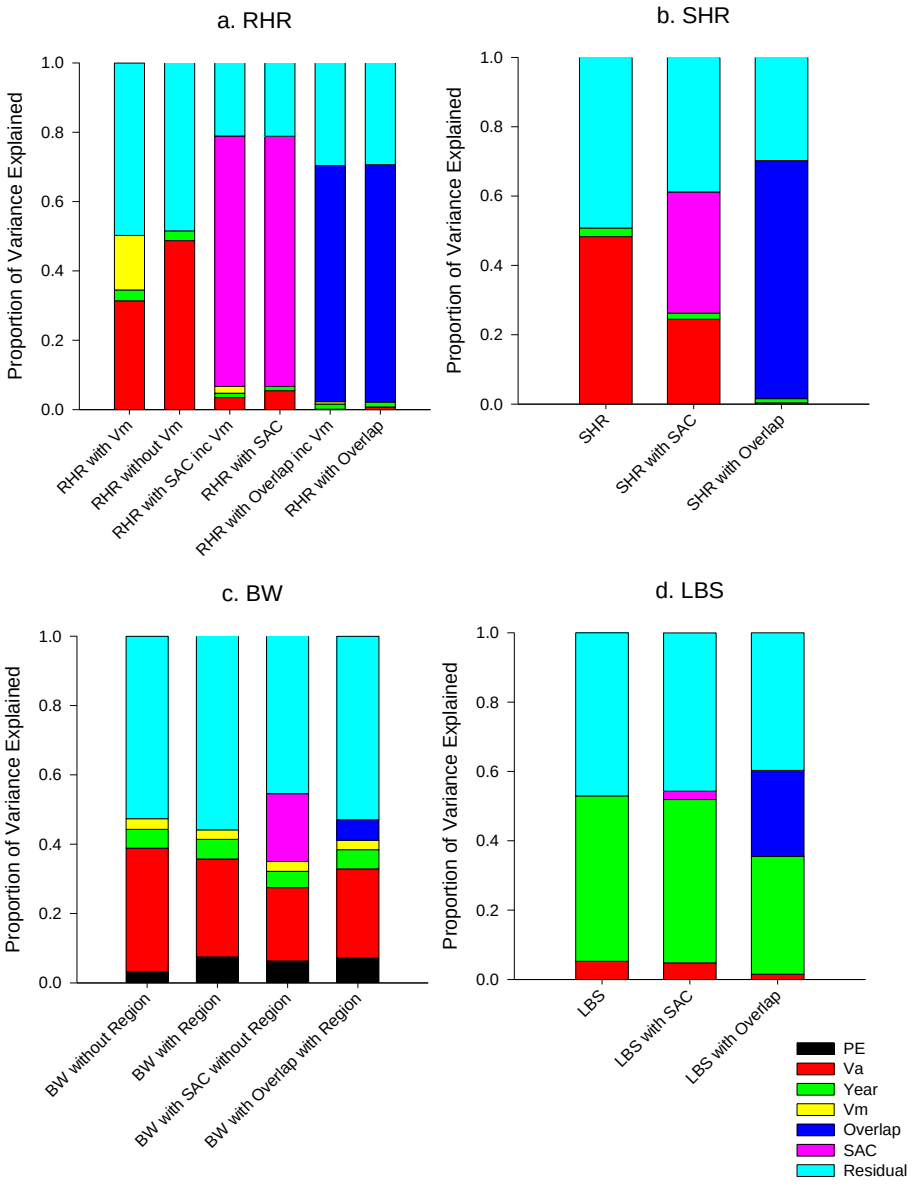
SAC

Finally, there was again improvement in models of LBS on fitting SAC (see table 6.1d), but the significance of this was marginal. Fitting maternal effects to models of LBS was not significant. Adding SAC to a model including additive genetic and birth year terms improved the model compared to no SAC (405.914 vs 401.979 on 3 degrees of freedom) but the p value (0.0488) was not significant after a Bonferroni correction for the seventeen tests (Bonferroni critical level of significance: 0.0023). Spatial variance explained only 2.41±1.61% of the variance, and this came mostly from the residual variance with very little change in heritability (see figure 6.4d). Interestingly, although the spatial variance was small, the estimated SAC parameter in the column direction was negative.

Home range overlap

Again, there was a significant home range overlap effect on LBS (see table 6.3d). Maternal effects were again not significant. The home range overlap term explained 24.85±4.86% of the variance in LBS, resulting in a decrease in the variance explained by the additive genetic, year and residual terms (see figure 6.4d). In particular, heritability decreased from 5.24±3.20% to 1.52±2.60%.

Figure 6.4: variance components. These are given for the 'best' (most parsimonious) models either without any spatial information, with SAC or with the S matrix ("overlap"): **a)** rut home range size, RHR, **b)** spring home range size, SHR, **c)** birth weight, BW and **d)** lifetime breeding success, LBS. For RHR, models are given with and without maternal effects (V_M), because the best model without spatial processes included maternal effects, but once spatial effects were included, maternal effects were not significant (see main text). For BW, the best model without spatial processes is given with and without region as a fixed effect, because the model with spatial autocorrelation did not include region as a fixed effect, but the model with overlap did.



Tables 6.1a-d: exploring the effects of spatial autocorrelation on variance components of **a)** rut home range, **b)** spring home range, **c)** birth weight and **d)** lifetime breeding success. "Comp" refers to the estimated variance component, given with standard error, as well as the proportion of variance explained for that term (and standard error). Italicised variance components are those which were bound at 0 or 1. Variance components are summed in the 'Sum' row for identification of models in which the sum of the variance components is an order of magnitude greater than in other models of the same trait. The variance components in such models are assumed to be poorly estimated. Additionally, the estimated spatial autocorrelation (φ) is given. P values appearing underneath log likelihoods are for a chi squared test of whether the log likelihood shown is significantly greater than the one immediately above (*i.e.* the change on adding an additional non-spatial variance component, for example, comparing a model with and without the additive genetic term). These tests are all on one degree of freedom unless stated. For tests of model improvement upon adding spatial terms, significance is denoted by asterisks, such that ****significant at p<=0.0001, ***significant at p<=0.001, **significant at p<=0.01, *significant at p<=0.05. Tests of adding an additional row OR column term are with two degrees of freedom, tests on adding an additional row and column term had three degrees of freedom. Log likelihoods in bold are the highest in the column considered. Log likelihoods in bold italics are the highest in each table. The model reported in the main text, and depicted in figure 6.4, which is "best" or most parsimonious model for each trait (discounting poorly fitting models, see main text) is in bold for identification. Note though For RHR, additional models are also displayed in figure 3 to illustrate the effect of adding SAC to models with and without maternal effects.

Table 6.1a. RHR

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
PE																				
PE	0.2638	0.0199	0.4799	0.0203	-82.75	0.1723	0.0142	0.0332	0.2954	-2.58	0.1390	0.0118	0.1093	0.1219	71.46	0.0618	0.0072	0.0566	0.0143	181.15
Column						4.7363	47.3632	0.9120	0.7838	****					****					****
Row											0.8536	1.4227	0.6711	0.3660						
C*R																0.7542	0.2557	0.6903	0.0725	
Residual	0.2859	0.0067	0.5201	0.0203		0.2848	0.0066	0.0548	0.4884		0.2793	0.0065	0.2196	0.2444		0.2765	0.0064	0.2531	0.0595	
Column φ						0.9869	0.1282									0.9558	0.0174			
Row φ											0.9439	0.0969				0.9023	0.0344			
Sum	0.5497					5.1934					1.2719					1.0925				
PE+ V_A																				
PE	0.0000	0.0000	0.0000	0.0000	13.09	0.0000	0.0000	0.0000	0.0000	70.88	0.0000	0.0000	0.0000	0.0000	129.37	0.0000	0.0000	0.0000	0.0000	216.59
V_A	0.2745	0.0220	0.4932	0.0216	(<0.00 001)	0.1903	0.0168	0.0035	0.0013	****	0.1456	0.0135	0.0948	0.1815	****	0.0696	0.0085	0.0570	0.0174	****
Column						54.2801	20.1785	0.9914	0.0032	(<0.00 001)					(<0.00 001)					(<0.00 001)
Row											1.1114	2.9247	0.7238	0.5283						
C*R																0.8758	0.3546	0.7171	0.0819	
Residual	0.2821	0.0066	0.5068	0.0216		0.2812	0.0065	0.0051	0.0019		0.2785	0.0064	0.1814	0.3470		0.2758	0.0063	0.2259	0.0657	
Column φ						*0.9990*	*0.0000*									0.9705	0.0131			
Row φ											0.9616	0.1044				0.9276	0.0296			
Sum	0.5566					54.7516					1.5355					1.2212				

Table 6.1a. RHR

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
PE+ V_A +Year																				
PE	0.0000	0.0000	0.0000	0.0000	87.42	0.0000	0.0000	0.0000	0.0000	145.27	0.0000	0.0000	0.0000	0.0000	**202.07**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	***288.66***
V_A	0.2694	0.0215	0.4875	0.0217		0.1865	0.0163	0.0247	0.3650	****	0.1431	0.0133	0.0941	0.1892	****	**0.0679**	**0.0083**	**0.0547**	**0.0171**	****
Year	0.0157	0.0045	0.0284	0.0080	(<0.00 001)	0.0159	0.0046	0.0021	0.0312	(<0.00 001)	0.0155	0.0045	0.0102	0.0207	(<0.00 001)	**0.0153**	**0.0044**	**0.0123**	**0.0051**	**(<0.00 001)**
Column						7.0873	118.1218	0.9379	0.9180											
Row											1.0976	3.0489	0.7218	0.5590						
C*R																**0.8958**	**0.3702**	**0.7218**	**0.0830**	
Residual	0.2675	0.0063	0.4841	0.0208		0.2666	0.0062	0.0353	0.5218		0.2644	0.0061	0.1739	0.3495		**0.2621**	**0.0060**	**0.2112**	**0.0633**	
Column φ						0.9920	0.1248									**0.9716**	**0.0129**			
Row φ											0.9630	0.1057				**0.9289**	**0.0295**			
Sum	0.5526					7.5563					1.5205					**1.2411**				
PE+ V_A +Year+ V_M																				
PE	0.0000	0.0000	0.0000	0.0000	**98.64**	0.0000	0.0000	0.0000	0.0000	**152.77**	0.0000	0.0000	0.0000	0.0000	196.38	0.0000	0.0000	0.0000	0.0000	281.58
V_A	0.1676	0.0200	0.3140	0.0323	****	0.1103	0.0153	0.0220	0.0081	****	0.1035	0.0138	0.0775	0.1287	****	0.0428	0.0086	0.0346	0.0124	****
Year	0.0166	0.0048	0.0311	0.0088	(<0.00 001)	0.0164	0.0047	0.0033	0.0015	0.00 001	0.0157	0.0045	0.0117	0.0197	(1)	0.0156	0.0045	0.0126	0.0053	(1)
V_M	0.0841	0.0178	0.1575	0.0313		0.0669	0.0146	0.0133	0.0055		0.0370	0.0104	0.0277	0.0464		0.0244	0.0074	0.0197	0.0085	
Column						4.5498	1.7041	0.9085	0.0314											
Row											0.9173	2.2374	0.6865	0.5194						
C*R																0.8933	0.3834	0.7225	0.0859	
Residual	0.2654	0.0063	0.4973	0.0210		0.2648	0.0063	0.0529	0.0180		0.2628	0.0062	0.1966	0.3259		0.2604	0.0061	0.2106	0.0655	
Column φ						0.9906										0.9697	0.0140			
Row φ											0.9588	0.1034				0.9350	0.0280			
Sum	0.5337					5.0564					1.3363					1.2365				

Table 6.1b. SHR

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
PE																				
PE	0.2834	0.0177	0.5433	0.0170	244.24	0.1536	0.0109	0.2358	0.0337	431.61	0.1317	0.0096	0.0073	0.0024	504.51	0.0443	0.0048	0.0081	0.0039	714.60
Column						0.2626	0.0858	0.4032	0.0789	****					****					****
Row											17.7192	5.7530	0.9798	0.0065						
C*R																5.2210	2.6638	0.9503	0.0241	
Residual	0.2382	0.0052	0.4567	0.0170		0.2351	0.0051	0.3610	0.0482		0.2337	0.0050	0.0129	0.0041		0.2289	0.0049	0.0417	0.0202	
Column φ						0.4141	0.1816									0.9641	0.0148			
Row φ											*0.9990*	*0.0000*				0.9964	0.0025			
Sum	0.5216					0.6513					18.0846					5.4942				
PE+ V_A																				
PE	0.0000	0.0000	0.0000	0.0000	494.58	0.0000	0.0000	0.0000	0.0000	529.98	0.0000	0.0000	0.0000	0.0000	690.22	0.0000	0.0000	0.0000	0.0000	802.61
V_A	0.2215	0.0153	0.4881	0.0187		0.1396	0.0109	0.2405	0.0332	****	0.1123	0.0091	0.1333	0.0272	****	0.0488	0.0053	0.0035	0.0010	****
Column					(<0.00 001)	0.2098	0.0716	0.3614	0.0791	(<0.00 001)					(<0.00 001)					
Row											0.5020	0.1588	0.5958	0.0768						
C*R																13.7867	3.6863	0.9804	0.0050	
Residual	0.2323	0.0050	0.5119	0.0187		0.2311	0.0049	0.3981	0.0499		0.2283	0.0048	0.2710	0.0513		0.2265	0.0047	0.0161	0.0042	
Column φ						0.4548	0.1729									0.9885	0.0041			
Row φ											0.9784					*0.9990*	*0.0000*			
Sum	0.4538					1.0353					0.8426					14.062				

Table 6.1b. SHR

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
PE+ V_A +Year																				
PE	0.0000	0.0000	0.0000	0.0000	**567.18**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**662.12**	0.0000	0.0000	0.0000	0.0000	757.38	0.0000	0.0000	0.0000	0.0000	***865.56***
V_A	0.2184	0.0150	0.4831	0.0189		**0.1396**	**0.0109**	**0.2448**	**0.0322**	********	0.1131	0.0091	0.0111	0.0036	****	0.0499	0.0054	0.0036	0.0010	********
Year	0.0113	0.0034	0.0249	0.0074	(<0.00 001)	**0.0101**	**0.0030**	**0.0177**	**0.0056**	**(<0.00 001)**	0.0099	0.0030	0.0010	0.0004	(<0.00 001)	0.0087	0.0026	0.0006	0.0003	(<0.00 001)
Column						**0.1994**	**0.0662**	**0.3495**	**0.0759**											
Row											9.8548	3.1893	0.9665	0.0106						
C*R																13.5066	3.6114	0.9800	0.0053	
Residual	0.2224	0.0048	0.4920	0.0182		**0.2213**	**0.0047**	**0.3880**	**0.0460**		0.2189	0.0047	0.0215	0.0067		0.2176	0.0046	0.0158	0.0041	
Column φ						**0.4186**	**0.1781**									0.9878	0.0043			
Row φ											*0.9990*	*0.0000*				*0.9990*	*0.0000*			
Sum	0.4521					**0.5704**					10.1967					13.7828				
PE+ V_A +Year+ V_M																				
PE	0.0000	0.0000	0.0000	0.0000	562.18	0.0000	0.0000	0.0000	0.0000	652.05	0.0000	0.0000	0.0000	0.0000	747.38	0.0000	0.0000	0.0000	0.0000	851.77
V_A	0.1934	0.0172	0.4370	0.0282		0.1187	0.0124	0.2101	0.0315	****	0.1083	0.0107	0.0108	0.0036	****	0.0438	0.0062	0.0032	0.0010	****
Year	0.0115	0.0035	0.0260	0.0078	(1)	0.0104	0.0031	0.0183	0.0059	(1)	0.0100	0.0030	0.0010	0.0004	(1)	0.0087	0.0026	0.0006	0.0003	(1)
V_M	0.0172	0.0086	0.0388	0.0196		0.0161	0.0072	0.0286	0.0131		0.0024	0.0046	0.0002	0.0005		0.0046	0.0036	0.0003	0.0003	
Column						0.2006	0.0660	0.3550	0.0758											
Row											9.7232	3.1672	0.9664	0.0106						
C*R																13.5108	3.6029	0.9802	0.0052	
Residual	0.2204	0.0048	0.4981	0.0186		0.2193	0.0048	0.3881	0.0463		0.2170	0.0047	0.0216	0.0068		0.2152	0.0046	0.0156	0.0041	
Column φ						0.4095	0.1765									0.9870	0.0046			
Row φ											*0.9990*	*0.0000*				*0.9990*	*0.0000*			
Sum	0.4425					0.5651					10.0609					13.7831				

Table 6.1c BW

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
PE																				
PE	0.6147	0.0556	0.4126	0.0248	-1249.58	0.5620	0.0534	0.3767	0.0267	-1242.47	0.5142	0.0497	0.0392	0.0233	-1224.75	0.5100	10.2700	0.2724	0.1016	-1224.52
Column						0.0547	0.0307	0.0367	0.0201	***					****					****
Row											11.7335	7.5700	0.8944	0.0616						(<0.00 001)
C*R																0.4908	0.7200	0.2621	0.2680	
Residual	0.8751	0.0311	0.5874	0.0248		0.8751	0.0311	0.5866	0.0259		0.8712	0.0309	0.0664	0.0385		0.8715	28.2100	0.4655	0.1692	
Column φ						-0.0715	0.5108									*0.9990*	*0.0000*			
Row φ											*0.9990*	*0.0000*				0.9764	25.5400			
Sum	1.4898					1.4918					13.1189					1.8723				
PE+ V_A																				
PE	0.0727	0.0568	0.0494	0.0393	-1205.57	0.0727	0.0572	0.0478	0.0402	-1205.56	0.1211	0.0585	0.0176	0.0149	-1200.03	0.1194	2.0400	0.0761	0.0395	-1200.15
V_A	0.5292	0.0920	0.3593	0.0517		0.5284	0.0920	0.3476	0.1074		0.4144	0.0858	0.0602	0.0449	**	0.4139	4.8200	0.2638	0.0664	*
Column					(<0.00 001)	0.0480	0.3997	0.0316	0.2595	(<0.00 001)					(<0.00 001)					(<0.00 001)
Row											5.4797	4.8067	0.7959	0.1441						
C*R																0.1659	0.6400	0.1057	0.1478	
Residual	0.8709	0.0308	0.5913	0.0276		0.8710	0.0308	0.5730	0.1538		0.8698	0.0308	0.1263	0.0885		0.8698	28.2800	0.5544	0.0936	
Column φ						*0.9990*	*0.0000*									*0.9990*	*0.0000*			
Row φ											*0.9990*	*0.0000*				0.9591	11.9200			
Sum	1.4728					1.5201					6.8850					1.5690				

Table 6.1c BW

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
PE+ V_A +Year																				
PE	0.0714	0.0562	0.0482	0.0385	-1161.32	(Not run)					0.1234	0.0579	0.0183	0.0153	-1155.56	0.1214	2.0900	0.0766	0.0394	-1155.68
V_A	0.5359	0.0918	0.3618	0.0513							0.4165	0.0855	0.0617	0.0456	**	0.4164	4.8600	0.2628	0.0698	**
Year	0.0745	0.0222	0.0503	0.0144	(<0.00 001)						0.0745	0.0221	0.0110	0.0083	(<0.00 001)	0.0745	3.3700	0.0470	0.0160	(<0.00 001)
Row											5.3409	4.6850	0.7909	0.1465						
C*R																0.1741	0.5900	0.1099	0.1658	
Residual	0.7994	0.0286	0.5397	0.0268							0.7980	0.0286	0.1182	0.0821		0.7981	27.920	0.5037	0.0958	
Column φ																*0.9990*	*0.0000*			
Row φ											*0.9990*	*0.0000*				0.9638	12.740			
Sum	1.4812										6.7443					1.5755				
PE+ V_A +Year+ V_M																				
PE	0.0486	0.0684	0.0327	0.0467	**-1076.04**	0.0497	0.0691	0.0334	0.0467	**-1075.89**	0.1116	0.0672	0.0090	0.0078	***-1065.70***	**0.1085**	**1.6100**	**0.0631**	**0.0432**	**-1065.79**
V_A	0.5299	0.0978	0.3561	0.0553		0.5167	0.0988	0.3474	0.0567		0.3621	0.0872	0.0293	0.0195	****	**0.3638**	**4.1600**	**0.2115**	**0.0760**	****
Year	0.0807	0.0247	0.0542	0.0159	(<0.00 001)	0.0807	0.0247	0.0542	0.0159	(<0.00 001)	0.0810	0.0248	0.0066	0.0045	(<0.00 001)	**0.0492**	**1.1500**	**0.0472**	**0.0190**	**(<0.00 001)**
V_M	0.0457	0.0432	0.0307	0.0288		0.0487	0.0431	0.0328	0.0289	(on 2 df)	0.0493	0.0429	0.0040	0.0043		**0.0812**	**3.2700**	**0.0286**	**0.0260**	
Column						0.0083	0.0170	0.0056	0.0115											
Row											10.9862	7.5248	0.8880	0.0689						
C*R																**0.3362**	**0.7000**	**0.1954**	**0.2244**	
Residual	0.7829	0.0291	0.5262	0.0274		0.7830	0.0291	0.5265	0.0275		0.7811	0.0290	0.0631	0.0385		**0.7814**	**0.0290**	**0.4542**	**0.1275**	
Column φ						0.4911	0.3300									***0.9990***	***0.0000***			
Row φ											*0.9990*	*0.0000*				**0.9673**	**17.510**			
Sum	1.4878					1.4871					12.3757					**1.7203**				

Table 6.1d LBS

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
V_A																				
V_A	0.0016	0.0081	0.0093	0.0457	288.84	0.0012	0.0079	0.0067	0.0442	293.37	0.0024	0.0085	0.0013	0.0047	298.92	0.0005	0.0080	0.0027	0.0444	296.81
Column						0.0047	0.0036	0.0269	0.0200	**					****					**
Row											1.6725	1.1003	0.9089	0.0548						
C*R																0.0149	0.0060	0.0848	0.0324	
Residual	0.1731	0.0116	0.9907	0.0457		0.1694	0.0114	0.9663	0.0488		0.1653	0.0117	0.0898	0.0543		0.1606	0.0115	0.9124	0.0543	
Column φ						-0.7546	0.2181									-0.6985	0.2098			
Row φ											*0.9990*	*0.0000*				0.4818	0.2604			
Sum	0.1891					0.1753					1.8402					0.176				
V_A+Year																				
V_A	0.0114	0.0070	0.0524	0.0320	401.98	0.0095	0.0068	0.0442	0.0309	**405.35**	0.0132	0.0073	0.0164	0.0149	**403.33**	**0.0105**	**0.0068**	**0.0481**	**0.0308**	***405.91****
Year	0.1039	0.0242	0.4772	0.0598		0.1014	0.0237	0.4698	0.0600	*	0.1025	0.0240	0.1272	0.0996		**0.1028**	**0.0240**	**0.4712**	**0.0600**	
Column					(<0.0001)	0.0030	0.0023	0.0140	0.0107	(<0.0001)					(<0.0001)					**(<0.0001)**
Row											0.5907	0.6089	0.7330	0.2035						
C*R																**0.0052**	**0.0035**	**0.0241**	**0.0161**	
Residual	0.1025	0.0084	0.4704	0.0657		0.1018	0.0082	0.4720	0.0650		0.0994	0.0084	0.1234	0.0948		**0.0996**	**0.0083**	**0.4566**	**0.0640**	
Column φ						-0.6879	0.2730									**-0.8359**	**0.1692**			
Row φ											*0.9990*	*0.0000*				**0.9253**	**0.0949**			
Sum	0.2178					0.2157					0.8058					**0.2145**				

Table 6.1d LBS

	No SAC					Column Processes					Row Processes					Column and Row Processes				
	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)	Comp	SE	Proportion Variance	SE	LogLik (P value)
V_A+Year+V_M																				
V_A	0.0085	0.0077	0.0435	0.0385	376.10	0.0073	0.0073	0.0375	0.0375	379.21	0.0108	0.0080	0.0110	0.0112	377.98	0.0081	0.0074	0.0414	0.0374	379.89
Year	0.0772	0.0201	0.3943	0.0639	(1)	0.0747	0.0195	0.3854	0.0638	*	0.0761	0.0199	0.0776	0.0606	(1)	0.0755	0.0198	0.3856	0.0640	(1)
V_M	0.0074	0.0050	0.0377	0.0260		0.0067	0.0049	0.0348	0.0256	(1)	0.0075	0.0050	0.0076	0.0076		0.0067	0.0049	0.0340	0.0252	
Column						0.0028	0.0024	0.0147	0.0124											
Row											0.7888	0.7304	0.8034	0.1474						
C*R																0.0052	0.0040	0.0267	0.0200	
Residual	0.1027	0.0090	0.5245	0.0710		0.1022	0.0089	0.5276	0.0707		0.0986	0.0091	0.1004	0.0761		0.1003	0.0090	0.5123	0.0702	
Column φ						-0.7750	0.2246									-0.8944	0.1293			
Row φ											*0.9990*	*0.0000*				0.9510	0.0744			
Sum	0.1958					0.1937					0.9818					0.2426				

Tables 6.2a-6.2d: inclusion of the S matrix in animal models of **a)** rut home range size, **b)** spring home range size, **c)** lifetime breeding success and **d)** birth weight. Models on the left of the table do not include the overlap term, but are given for comparison with the model on the right of the table, which does. P values in the final column are given for a chi squared test comparing the log likelihood of the model to that of the model to the left (i.e. with and without overlap), on one degree of freedom. P values appearing underneath log Likelihoods are for a chi squared test of whether the log likelihood shown is significantly greater than the one immediately above (*i.e.* the change on adding an additional non-spatial variance component, for example, comparing a model with and without the additive genetic term). These tests are all on one degree of freedom unless stated. Similarly to the previous table, the best model is identified in bold.

6.2a: RH**R**	Without Overlap					With Overlap					
	Component	SE	Proportion Variance	SE	LogLik (P value)	Component	SE	Proportion Variance	SE	LogLik (P value)	P value
PE+Residual											
PE	0.2638	0.0199	0.4799	0.0203	-82.7479	0.0004	0.0024	0.0005	0.0026	445.5630	<0.0001
Overlap						0.6639	0.1287	0.7056	0.0410		
Residual	0.2859	0.0067	0.5201	0.0203		0.2766	0.0063	0.2940	0.0404		
Sum	0.5497					0.9409					
PE+V_A+Residual											
PE	0.0000	0.0000	0.0000	0.0000	13.0930	0.0000	0.0000	0.0000	0.0000	450.0840	<0.0001
V_A	0.2745	0.0220	0.4932	0.0216	(<0.0001)	0.0057	0.0025	0.0064	0.0031	(0.0026)	
Overlap						0.6091	0.1206	0.6861	0.0433		
Residual	0.2821	0.0066	0.5068	0.0216		0.2730	0.0061	0.3075	0.0420		
Sum	0.5566					0.8878					
PE+V_A+Year+Residual											
PE	0.0000	0.0000	0.0000	0.0000	87.4218	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**511.8140**	**<0.0001**
V_A	0.2694	0.0215	0.4875	0.0217	(<0.0001)	**0.0067**	**0.0026**	**0.0076**	**0.0032**	**(<0.0001)**	
Year	0.0157	0.0045	0.0284	0.008		**0.0128**	**0.0037**	**0.0144**	**0.0046**		
Overlap						**0.6080**	**0.1202**	**0.6846**	**0.0434**		
Residual	0.2675	0.0063	0.4841	0.0208		**0.2605**	**0.0059**	**0.2934**	**0.0400**		
Sum	0.5526					**0.8881**					
PE+V_A+Year+V_M+Residual											
PE	0.0000	0.0000	0.0000	0.0000	98.6448	0.0000	0.0000	0.0000	0.0000	489.5240	<0.0001
V_A	0.1676	0.0200	0.3140	0.0323	(<0.00001)	0.0011	0.0029	0.0012	0.0033	(1)	
Year	0.0166	0.0048	0.0311	0.0088		0.0131	0.0039	0.0149	0.0048		
V_M	0.0841	0.0178	0.1575	0.0313		0.0060	0.0030	0.0068	0.0036		
Overlap						0.5981	0.1203	0.6812	0.0446		
Residual	0.2654	0.0063	0.4973	0.021		0.2598	0.0060	0.2959	0.0409		
Sum	0.5337					0.8780					

6.2b: SHR	Without Overlap					With Overlap					
	Component	SE	Proportion Variance	SE	LogLik (P value)	Component	SE	Proportion Variance	SE	LogLik (P value)	P value
PE+Residual											
PE	0.2834	0.0177	0.5433	0.0170	244.2410	0.0000	0.0000	0.0000	0.0000	1179.6300	<0.0001
Overlap						0.5030	0.0886	0.6984	0.0375		
Residual	0.2382	0.0052	0.4567	0.0170		0.2172	0.0043	0.3016	0.0375		
Sum	0.5216					0.7202					
PE+V_A+Residual											
PE	0.0000	0.0000	0.0000	0.0000	494.5810	0.0000	0.0000	0.0000	0.0000	1181.7200	<0.0001
V_A	0.2215	0.0153	0.4881	0.0187	(<0.0001)	0.0021	0.0014	0.0030	0.0020	(0.0409)	
Overlap						0.4809	0.0863	0.6883	0.0390		
Residual	0.2323	0.0050	0.5119	0.0187		0.2157	0.0044	0.3087	0.0384		
Sum	0.4538					0.6986					
PE+V_A+Year+Residual											
PE	0.0000	0.0000	0.0000	0.0000	567.1770	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**1246.0000**	**<0.0001**
V_A	0.2184	0.0150	0.4831	0.0189	(<0.0001)	**0.0025**	**0.0014**	**0.0037**	**0.0021**	**(0)**	
Year	0.0113	0.0034	0.0249	0.0074		**0.0083**	**0.0025**	**0.0120**	**0.0038**		
Overlap						**0.4788**	**0.0860**	**0.6868**	**0.0392**		
Residual	0.2224	0.0048	0.4920	0.0182		**0.2075**	**0.0042**	**0.2976**	**0.0370**		
Sum	0.4521					**0.6972**					
PE+V_A+Year+V_M+Residual											
PE	0.0000	0.0000	0.0000	0.0000	562.1840	0.0000	0.0000	0.0000	0.0000	1218.7900	<0.0001
V_A	0.1934	0.0172	0.4370	0.0282	(1)	0.0019	0.0018	0.0027	0.0026	(1)	
Year	0.0115	0.0035	0.0260	0.0078		0.0081	0.0024	0.0115	0.0037		
V_M	0.0172	0.0086	0.0388	0.0196		0.0001	0.0015	0.0001	0.0021		
Overlap						0.4872	0.0876	0.6930	0.0388		
Residual	0.2204	0.0048	0.4981	0.0186		0.2058	0.0043	0.2927	0.0368		
Sum	0.4425					0.7030	0.0000				

6.2c: BW	WithoutOverlap					WithOverlap					
	Component	SE	Proportion Variance	SE	LogLik (P value)	Component	SE	Proportion Variance	SE	LogLik (P value)	P value
PE+Residual											
PE	0.5206	10.5000	0.3727	0.0250	-1229.7400	0.5039	0.0489	0.3457	0.0291	-1225.6700	0.0043
Overlap			0.6273	0.0250		0.0811	0.0615	0.0557	0.0399		
Residual	0.8764	28.1800				0.8727	0.0310	0.5986	0.0331		
Sum	1.3970					1.4578					
PE+V_A+Residual											
PE	0.1120	1.9400	0.0794	0.0416	-1202.9100	0.1108	0.0000	0.0757	0.0402	-1200.8100	0.0404
V_A	0.4261	4.9700	0.3021	0.0527	(<0.0001)	0.4147	0.0851	0.2835	0.0526	(<0.0001)	
Overlap						0.0667	0.0612	0.0456	0.0403		
Residual	0.8726	28.2500	0.6185	0.0276		0.8703	0.0308	0.5951	0.0348		
Sum	1.4107					1.4625					
PE+V_A+Year+Residual											
PE	0.1103	1.9500	0.0778	0.0407	-1158.9600	0.1101	0.0000	0.0742	0.0390	-1156.2000	0.0188
V_A	0.4315	5.0600	0.3043	0.0521	(<0.0001)	0.4172	0.0846	0.2811	0.0520	(<0.0001)	
Year	0.0747	3.3500	0.0527	0.0151		0.0758	0.0226	0.0511	0.0147		
Overlap						0.0824	0.0698	0.0555	0.0447		
Residual	0.8016	27.8900	0.5653	0.0270		0.7985	0.0286	0.5381	0.0345		
Sum	1.4182					1.4839					
PE+V_A+Year+V_M+Residual											
PE	0.1058	1.5700	0.0752	0.0487	-1071.4100	**0.0815**	**0.0000**	**0.0722**	**0.0463**	**-1068.7400**	**0.0208**
V_A	0.3970	4.4800	0.2821	0.0560	(<0.0001)	**0.0402**	**0.0406**	**0.2569**	**0.0554**	**(<0.0001)**	
Year	0.0801	3.2600	0.0569	0.0167		**0.1067**	**0.0671**	**0.0551**	**0.0163**		
V_M	0.0386	0.9400	0.0274	0.0290		**0.3797**	**0.0881**	**0.0272**	**0.0273**		
Overlap						**0.0875**	**0.0742**	**0.0592**	**0.0476**		
Residual	0.7856	26.9000	0.5584	0.0278		**0.7821**	**0.0291**	**0.5293**	**0.0358**		
Sum	1.4070					**1.4776**					

6.2d: LBS Without Overlap With Overlap

	Component	Comp/SE	Proportion	SE	LogLik	Component	SE	Proportion	SE	LogLik	
V_A+Residual											
V_A	0.0016	0.0081	0.0093	0.0457	288.844	0.0048	0.0068	0.0279	0.0369	429.9890	<0.0001
Overlap						0.0705	0.0147	0.4121	0.0573		
Residual	0.1731	0.0116	0.9907	0.0457		0.0958	0.0082	0.5600	0.0646		
Sum	0.1747					0.1710					
V_A+Year+Residual											
V_A	0.0114	0.007	0.0524	0.0320	401.979	**0.0028**	**0.0000**	**0.0152**	**0.0260**	**500.5650**	**<0.0001**
Year	0.1039	0.0242	0.4772	0.0598	(<0.0001)	**0.0617**	**0.0150**	**0.3395**	**0.0580**	**(<0.0001)**	
Overlap						**0.0452**	**0.0099**	**0.2485**	**0.0486**		
Residual	0.1025	0.0084	0.4704	0.0657		**0.0721**	**0.0063**	**0.3968**	**0.0527**		
Sum	0.2178					**0.1817**					
V_A+Year+Residual											
V_A	0.0085	0.0077	0.0435	0.0385	376.095	0.0000	0.0000	0.0000	0.0000	468.8920	<0.0001
Year	0.0772	0.0201	0.3943	0.0639	(1)	0.0400	0.0109	0.2447	0.0533	(1)	
V_M	0.0074	0.005	0.0377	0.026		0.0026	0.0034	0.0158	0.0212		
Overlap						0.0448	0.0102	0.2746	0.0524		
Residual	0.1027	0.009	0.5245	0.071		0.0759	0.0057	0.4649	0.0524		
Sum	0.1958					0.1633					

6.5 Discussion

Whenever individuals are philopatric, such that relatives tend to associate together, genetic and environmental causes of similarity are potentially confounded. In this study, we have demonstrated upward biases in estimates of heritability of rut home range size, spring home range size, birth weight and lifetime breeding success in a wild population of red deer arising from shared environment effects between individuals. The extent to which spatial effects explained the variance in the four traits considered differed greatly between traits, ranging from 2-70%.

In both home range size traits, there was substantial variance explained both by location and home range overlap, accounting for between 35-70% of the variance. Both traits were positively spatially autocorrelated, indicating individuals with nearby average lifetime locations were likely to have similar-sized home ranges. This is not surprising, as home ranges are an example of a plastic trait which is likely to be dependent upon an underlying spatially autocorrelated variable, food availability. Additionally, the substantial component of variance explained by home range overlap in these traits shows that not just an individual's average location but also the physical area covered by their home range has an important influence on home range size: again, this is not surprising, as the size of an individual's home range results from the trade-off between the resources gained by ranging further and the cost of doing so; therefore, an individual in a better environment is likely to have a smaller home range size (McNab 1963, Moyes 2007).

In our analysis, for home range traits, including SAC or home range overlap in animal models resulted in a substantial reduction in estimates of additive genetic variance. This demonstrates that for these traits, if spatial processes are not accounted for, environmental and genetic similarity between individuals is confounded, and therefore the heritability of these traits, and potentially the response to selection, is greatly overestimated. Further, for rut home range size, when either SAC or home range overlap was included in the model, maternal effects were no longer significant; therefore what appear to be maternal effects are

probably a result of matrilineal relatives associating together and using similar home ranges. Although here we have not separated maternal environmental and genetic effects, given total heritability depends upon both direct and maternal genetic variance (Wilham 1972), our findings suggest it will be important to account for confounding effects of environmental similarity between maternal relatives on estimates of maternal genetic effects.

We also found significant spatial effects on offspring birth weight. The variance explained by home range overlap was small (around 6%) for birth weight, but was significant. Furthermore this trait appeared to be positively spatially auto-correlated (although the variance associated with this was estimated with high uncertainty) indicating individuals whose average lifetime locations were similar had offspring of similar weights. Accounting for this shared environment effect led to a small reduction in estimates of heritability of birth weight slightly.

Home range overlap explained around 25% of the variance in lifetime breeding success. Although little genetic variance existed in lifetime breeding success anyway, including home range overlap resulted in a decrease in heritability of this trait also from 5.24±3.20% to 1.52±2.60%, again indicating that the heritability of this trait was upwardly biased by relatives experiencing shared environments. This indicates that the area in which a female's home range is located has significant effects on fitness, suggesting there is spatial heterogeneity in fitness across the study area. Conradt *et al.* (1999) found evidence for spatial variance in lifetime fitness over large spatial scales (at the level of region) in this population, but at finer scales there was no correlation between lifetime reproductive success and use of *Agrostis/Festuca* (see figure 1) after accounting for local population density. The variance at the level of region should be accounted for in our models by our inclusion of region as a fixed effect. However, later analysis (McLoughlin *et al.* 2006) identified significant spatial heterogeneity in fitness linked to the relationships between use of *Agrostis/Festuca* grassland, local population density and lifetime reproductive success, and suggested this heterogeneity could be maintained by social constraints to dispersal preventing females from moving to more productive areas. Although not significant in the final model, we found

fitting SAC processes to models of lifetime breeding success did reveal interesting patterns in spatial heterogeneity of LBS: although SAC was positive in the row direction for this trait, SAC was negative in the column direction. Negative SAC indicates that individuals with similar average lifetime locations were less likely to have similar trait values. In ecology, negative SAC is indicative of competition, such that individuals with high trait values depress the trait values of neighbours (Dukowtski 2002, Haining 2004). We did not fit local population density (*i.e.* competition) to this model, as this was a lifetime measure of fitness and local population density varies annually, although to some extent it should be reflected by the region term. McLoughlin *et al.* (2006) found evidence for negative effects of competition on LRS at high density, such that at very high densities, selection for *Agrostis/Festuca* was negatively correlated with LRS. Because of the distribution of females in the study area, the majority of information in column processes comes from the North of the study area, moving East from Kilmory to Shamhan Insir (see figure 1). This is an area of higher density compared to the south of the study area (McLoughlin *et al.* 2006), and it seems likely that high population density results in greater competition between individuals in this direction.

The effect of including the S matrix differed from that produced by fitting SAC processes. Fitting home range overlap reduced estimates of additive genetic variance in home range sizes more than SAC. Further, inclusion of the S matrix was significant in models of birth weight and LBS, whereas fitting SAC was not significant in models of LBS and produced poor estimates of spatial variance for birth weight. Fitting the matrix of home range overlap is arguably a more appropriate way to deal with causes of environmental similarity between relatives because patterns of space use, as indicated by home range overlap, are more likely to accurately describe the similarity of the environment two individuals experience, in terms of available food and shelter, and the energy they have to expend to acquire these. Because we used a home range overlap that included information on the utilization distribution of home ranges, *i.e.* the amount individuals actually use different parts of the home range, the home range overlap matrix gives us a very accurate measure of extent to which individuals experience

similar environmental conditions. In contrast, using an average location (as for SAC) is a cruder measure of the environment an individual experiences, not least because the error on the estimate of average location is likely to vary between individuals depending upon the extent to which animals range around that average location. Even fitting a home range centre instead of an average coordinate is unlikely to deal with the problem of individuals having multiple centres of activity within a lifetime home range (Rhodes *et al.* 2005). Further, we found that models including SAC were not necessarily stable in the parameters they estimated, nor their likelihood of converging, particularly where traits contained little spatial variance; in contrast, models using the double matrix approach appeared to converge to a more stable solution.

To some extent, spatial processes at global scales can be fitted as fixed effects, such as region, or local population density, to account for spatial effects. Region and row/column processes were clearly confounded in models of birth weight, indicating that region probably explains at a global level the SAC we have observed in that trait. However, fitting home range overlap explained a significant amount of the variance in this trait even when region was fitted, and doing so still resulted in a slight decrease in the estimate of genetic variance. Further, whilst spatial effects on birth weight may be partly accounted for by region, this was clearly not true for the other three traits. Fitting fixed effects to account for global processes is only possible where prior knowledge exists about global spatial processes that are occurring, such as a distinct change in habitat type, and they cannot account for fine scale heterogeneity. Therefore, fitting such effects is clearly beneficial where information exists but is unlikely to be sufficient to address the problem of shared environment.

It is striking that we found such strong effects of home range overlap on the traits considered despite the existence of certain limitations in our home range overlap matrix. For example, the matrix uses lifetime locations, and includes nothing about when individuals existed: it therefore assumes individuals with similar home ranges separated by as much as 30 years experience the same environmental conditions. Ideally therefore, temporal information on overlap of individuals in

time as well as space would be incorporated, and/or the matrix would be constructed on an annual basis. However, producing home range overlap matrices for large populations is not trivial, and the trade-off from information gained by calculating overlap on an annual basis and the computational time to do so and to fit the appropriate information to the animal model is potentially prohibitive.

In general however, this 'double matrix' technique - fitting both genetic relatedness and environmental similarity - offers exciting possibilities for separating other causes of similarity between individuals. Beyond spatial analysis, the multi-matrix method could also be used to assess the variance explained in traits by association between individuals. The use of social network analysis has recently become very popular in behavioural ecology to identify and quantify the interactions between individuals and the extent to which individuals associate (Wey *et al.* 2008). This has been used to describe social structure and predict patterns of cooperation in guppies (Croft *et al.* 2004, Croft *et al.* 2006), and spatial-association networks in bats are thought to be important in not just social life but also epidemiology (Rhodes *et al.* 2006, Wey *et al.* 2008). Moving beyond simply identifying social structure, the fitness correlates of social relationships are not well known (but see Silk *et al.* 2003, Frere *et al.* 2010). In a recent attempt to investigate this, Frere *et al.* (2010) stated that a matrix of genetic relatedness and social interactions could not be fitted simultaneously within an animal model; however, it appears from our study that this would be perfectly possible, and would allow researchers to identify the relative importance of these components of individual similarity without recourse to the misuse of BLUPs (Hadfield *et al.* 2010). By using the multi-matrix technique, a matrix of interactions between individuals, *i.e.* an association matrix (Whitehead 2008), could be fitted to explain variance in traits including fitness. This could potentially even be extended to an "ultimate animal model", in which similarity between individuals in wild populations was separated into relatedness, shared environment and social associations. It should be noted however that doing so would require sufficient data and also sufficient independence between the matrices to allow their separation.

Conclusions

In the past decade, there has been an important advance in the wild animal quantitative genetic literature in understanding the need to account for shared environment and spatial autocorrelation in estimates of heritability and selection (see Kruuk and Hadfield 2007). However, with one exception (Van der Jeugd and McCleery 2002), there has been little attempt to model such effects in wild animal populations other than through the inclusion of brood or maternal effects in animal models. Here, for the first time in a wild animal population, we have made use of spatial autoregressive techniques widely used in agricultural and forestry literature to account for spatial autocorrelation in traits in wild animals. Further, we have used a novel method - fitting a matrix of home range overlap - to simultaneously estimate genetic and environmental similarities between individuals. In doing so, we have substantially reduced the bias due to environmental causes of similarity in our estimates of heritabilities of the traits considered. We therefore suggest the implementation of such techniques is vital in animal models of any wild trait likely to be affected by environmental variation, and further work should be directed at the refinement of such techniques for use in, for example, identifying genetic correlations and estimating selection.

Chapter 7:

General Discussion

The aim of this thesis was to investigate the causes and consequences of sexual selection in wild red deer. Specifically, in this thesis, I have i) documented the movements of females during the breeding season and assessed evidence as to whether these are expressions of mating preferences, ii) shown how male competition and variance in female oestrus date are associated with between-year variation in the variance of male reproductive success, iii) found substantial between and within individual variation in male acoustic signalling, iv) documented surprising rates of female re-mating with same male and intra-lineage polygyny, and concomitant effects on co-ancestry and inbreeding and v) used novel methods to account for shared environment effects in estimates of trait heritability of home range size, birth weight and lifetime breeding success.

A detailed discussion of each these findings with respect to the relevant literature is given at the end of each data chapter. Rather than repeat these sections, in this chapter I attempt to explain briefly how my findings contribute to our overall understanding of sexual selection. Moreover, I highlight future directions for study, particularly in the red deer of Rum, which arise from my findings. I discuss some of the limitations to the data used which are relevant to the work presented. Finally, I discuss the importance of studying sexual selection in wild populations.

7.1 The process of sexual selection in red deer

7.1.1 The interplay between male competition and female choice

Outwardly, the mating system of the red deer is dominated by competition between males, mediated by body size, fighting ability, antler mass and acoustic signals (roars), which reflect these traits, and this competition results in large variance in male mating success (Clutton-Brock *et al.* 1979, Clutton-Brock 1989, Kruuk *et al.* 2002b, Reby and McComb 2003a). However, it has long been noted that other dimensions exist to rut behaviour within the study system. For example, females have been documented to avoid mating with young males, and young males attempt to sneak into harems defended by adult males to gain copulations (Clutton-Brock *et al.* 1982). Work on other red deer populations, including those in captivity, has revealed that females may show preferences for antler

traits (Bartos *et al.* 2007, Malo *et al.* 2005), properties of the male roar (Charlton *et al.* 2007a, Charlton *et al.* 2007b, McComb 1991, Reby *et al.* 2001, Reby and McComb 2003a, Reby *et al.* 2010 but see Charlton *et al.* 2008), and even male territory (Carranza 1995).

In this thesis, I have made a substantial contribution to our understanding of the role of female behaviour in the mating system of the red deer, presenting evidence of female behaviour during the rut which is not necessarily explained by male-male competition for access to mates (Chapter 2, Chapter 5). I have demonstrated that females in oestrus move substantial distances between harems, and that a third of such movements result in the novel male gaining paternity of the female's offspring in that season (Chapter 2). However, I found no evidence that the tendency of females to move to older males or larger harems is related to oestrus, nor any evidence females move to less related males. I also show that females mate with the same male in multiple years and with the same male as their female relatives more than expected both under random mating and as a result of temporal, spatial and male-male competition constraints on mating behaviour. Taken together, these behaviours clearly have important effects on the genetic structure of the population (see Chapter 5) and have the potential to impact on the outcome of male-male competition in terms of male reproductive success. However, much remains to be uncovered about whether the mating behaviours observed in females are adaptive, rather than a consequence of male harassment or constraints on the mating system (other than those modelled in the simulations presented in Chapter 5). For example, the observed intra-lineage polygyny may be a consequence of mate copying: it would therefore be interesting to explore whether unrelated females which share home ranges tend to mate with the same male as often as related females which share home ranges. Further, the link between female movements and the mating behaviours described in Chapter 5 remain to be investigated; given that spatial constraints did not explain all the observed re-mating and intra-lineage polygyny, movements of females when in oestrus may play a role in facilitating these behaviours.

It has been argued that empirical studies of sexual selection typically focus on either male-male competition or female choice, and rarely attempt to combine the two to give a total description of sexual selection in a population (Hunt *et al.* 2009). Because male-male competition and female choice may be either reinforcing or opposing, failure to consider both mechanisms together can misinform our understanding of how selection acts on sexual traits (Qvanstrom and Forsgren 1998, Wong and Candolin 2005, Hunt *et al.* 2009).

The behaviours I have documented have the potential to either reinforce or weaken sexual selection on traits from male-male competition, depending on whether the same male phenotypes are successful in both male-male competition and as a result of female behaviours. Given that throughout the rut, females preferentially moved to older males and males with larger harems, the findings of Chapter 2 suggest that female movements may reinforce the outcome of male competition on male reproductive success. Further, including the observed age-specific male reproductive success in simulations of the mating system increased the extent of re-mating and intra-lineage polygyny simulated (see Chapter 5), suggesting these behaviours might reinforce the effects of male competition. As well as observational study to determine the extent to which female movements are due to female decisions, rather than harassment by males, future work should address how the female behaviours we have documented affect the distribution of male reproductive success compared to that expected from male competition, in order to better understand how the processes interact to result in sexual selection in this population.

7.1.2 Environmental effects on sexual selection

7.1.2.1 Environmental effects on the strength of selection

In this thesis I have shown that variance in male reproductive success, and so the opportunity for sexual selection, depends upon the interaction between the number of males which temporarily immigrate into the population to rut in a year, and the temporal synchrony of female oestrus. The effects of ecological variation on mating systems have been given substantial attention, both theoretically (Emlen and Oring 1977, Kokko and Rankin 2006) and empirically, in both lab and wild studies (Clutton-Brock and Harvey 1978, Cockburn *et al.* 2008, McLain 1993, Jones *et al.* 2004, Punzalan *et al.* 2010). In recent years this attention has widened to include variation not just between but within seasons (Reichard *et al.* 2008, Kasumovic *et al.* 2008). Chapter 2 makes an important contribution to this field by simultaneously considering the effect of multiple ecological parameters on between-season variation in male reproductive success. This has allowed me to a) identify that is the number of immigrant males that has the greatest impact on variance in male reproductive success, rather than sex ratio (as might be predicted Emlen and Oring 1977), and b) demonstrate that the interaction between this and variance in female oestrus date is the main driver of variance in male reproductive success.

Chapter 2 also suggests that selection on male sexual traits, particularly rut start date, might be affected by the biotic environment, and therefore that directional selection on male traits is likely to be temporally heterogeneous between seasons because of changes in social environmental conditions. This is a potentially important mechanism in the maintenance of genetic variation. Under social conditions which are favourable for less competitive males (low numbers of immigrant males rutting and highly synchronised female oestruses), selection on rut start date is weaker, and so we would predict genetic variation for rut start date to be greater. In general, favourable conditions are associated with higher additive genetic variance in traits (Charmantier and Garant 2005), although to date the traits considered are mostly non-sexual, and the conditions considered are usually environmental rather than to do with the social environment (Ingleby *et al.* 2010). Now that the relevant demographic and ecological conditions affecting variance in male reproductive success, and so potentially sexual selection, have been identified for the red deer of Rum, further study of this population should be directed at explicitly testing for genotype by environment interactions in sexual traits with changes in social environment. Further, my findings suggest it would be fruitful to undertake investigation of the role of indirect genetic effects on competition between red deer males, in relation to how the outcome of competition between males is affected by the distribution of competitive abilities amongst males in each breeding season (Wolf *et al.* 1998, Moore *et al.* 2002, Wilson *et al.* 2011).

7.1.2.2. Intra-individual variation in sexual signals

It has been argued that the formant frequencies of the roars of red deer males are an acoustic cue to body size used in both male-male and male-female interactions. I have shown that this important male sexual signal not only varies between but also within individuals. My results also suggest the within-individual variation observed is related to how individuals respond to changes in social context. The pattern of selection on this trait is likely to be affected by this intra-individual variation, with selection potentially acting on plasticity itself.

The findings I present are limited by sample size and by being documented in only one breeding season. Despite this, the intra-individual variation we have observed provokes interesting questions as to the reliability of formant frequencies as a sexually selected signal. Unreliability of sexual signals *between* generations resulting from a dependency of the signal on varying environmental conditions has been implicated as a difficulty of

using genotype by environment interactions in sexual signals to resolve the lek paradox (Greenfield and Rodriguez 2004). This is because male offspring might experience a different environment to that in which their father was successful. Unreliability of sexual signals *within* seasons due to intra-individual variation in the signal could undermine the utility of the signal if it results in the receiver gaining uninformative information. For example, if a large male produces a roar which underestimates his body size, it might invite a smaller, weaker male to attempt to fight, which is energetically costly for both males and potentially dangerous for the smaller male. It is true that as long as on average the relationship between male reproductive success and the signal are maintained then there will still be positive directional selection on the trait. However, my findings illustrate that characterizing an individual's trait at any one point could a) misinform as to how selection will act upon that individual and b) miss a substantial amount of variation which may be relevant to the evolution of that trait.

Clearly, we have much still to understand about why male red deer vary so much in the formant frequencies they produce when roaring under different social and environmental conditions. The current evidence that females chose males for this trait in the wild is also equivocal at best (see Charlton *et al.* 2008). A clearer understanding of this trait in the wild, and therefore how selection acts upon the trait, is needed to assess the importance of the observed variation to the evolution of roaring as a sexual signal. A vital next step would be to try and investigate further how much of the observed intra-individual variation in formant frequencies results from plasticity rather than noise or mistakes in signalling. In particular, we lack understanding of whether the intended receiver is other males or females; knowing this would aid interpretation of the observed variation and its likely function (if any). We therefore require more targeted recording of focal individuals under different contexts, and further, doing so over multiple breeding seasons to investigate whether differences between individuals in plasticity are consistent over multiple breeding seasons. This could then be analysed with a behavioural reaction norm approach to identify repeatability of plasticity in behaviour, an important test of how plasticity and animal personality are linked (Dingemanse *et al.* 2009). For example, either through male-male competition or female choice, selection might favour males who are most consistent in their formant frequencies (Schuett *et al.* 2010).

7.2 Philopatry and inbreeding

In chapter 4, I demonstrate that the extent to which females re-mate with the same male, and female relatives mate with the male, are associated with a population genetic structure in which pairs of individuals are more related than expected by chance, and there is more inbreeding than expected. Relatedness and inbreeding were not only higher than expected by chance, but also higher than expected after taking into account fidelity of rut timing and rut location, and the association between male age and male reproductive success.

These findings have two important implications in relation to our understanding of sexual selection, both in the study population and more widely. Firstly, they highlight how mating patterns could increase kinship, providing opportunities for kin selection in species without strongly defined social groups. In horseshoe bats, increased co-ancestry amongst females arising from intra-lineage polygyny has been implicated in the evolution of co-operative behaviours amongst females roosting in the same groups (Rossiter *et al.* 2005). Similarly, in red deer, increased co-ancestry between females within a matriline may result in greater tolerance amongst groups of grazing hinds. Kin structure of populations may be important in population dynamics (*e.g.* Lambin and Krebs 1993). In the deer, density-dependent effects on competition could be mediated by tolerance of relatives grazing locally. Future studies should therefore investigate the relationship between co-ancestry and demography, perhaps by comparison of the extent of intra-lineage polygyny within different matrilines and their reproductive success.

Secondly, we have documented a surprising amount of inbreeding. The costs of inbreeding in wild populations, *i.e.* inbreeding depression, have been widely shown (reviewed in Keller and Waller 2002), generating the expectation that animals should avoid mating with relatives. In polygynous mammals, such as the red deer, male dispersal coupled with female philopatry is expected to prevent relatives from mating. However, I have shown that male location is not random with respect to relatedness; as a result females encounter male relatives and mate with them. The resulting inbreeding is then reinforced by the processes of intra-lineage polygyny and re-mating (*e.g.* see figure 5.7). Although this result is surprising, it is not wholly unexpected with respect to theoretical investigation of this problem. It has been shown that inclusive fitness benefits to females can favour inbreeding, depending upon the extent to which it reduces the mating opportunities of the male relative, and the costs in terms of offspring viability (Parker 1979, Smith 1979, Waser *et al.* 1986, Kokko and Ots 2006). Clearly, further work is

needed in natural populations to link the potential costs of inbreeding depression with the extent to which individuals actually avoid inbreeding. In the red deer, a measure of inbreeding depression, d^2, has been linked to birth weight and male calf survival (Coulson *et al.* 1998, Coulson *et al.* 1999, Slate and Pemberton 2002) and heterozygosity has been shown to be positively correlated with lifetime breeding success (Slate *et al.* 2000). Further work is underway to calculate the costs of inbreeding in terms of neonatal traits using pedigree inbreeding coefficients. This has shown significant inbreeding depression in offspring birth weight and first year survival, but not birth date. The effects of first year survival appear to be particularly strong: offspring with f=0.25 show a 66% reduction in survival compared to outbred offspring (Walling *et al.* in prep). Once this work is complete, it will be informative to link the expected costs of particular mating events with the extent to which they occur more frequently than expected by chance (or after temporal and spatial constraints are considered). Further, such parameters could be used to formally test the predictions of theoretical studies (Smith 1979, Waser *et al.* 1986, Kokko and Ots 2006) as to when the inclusive fitness benefits of inbreeding will outweigh the costs. However, this investigation would require more extensive knowledge of the costs of inbreeding on not just neonatal but also lifetime fitness.

Future work should also be directed at identifying why male deer return to rut in the same locations as their relatives. In lekking species, male relatives gain benefits by breeding in on the same lek because of an increase in the number of females joining the lek per male, and because even unsuccessful males on such leks gain inclusive fitness benefits from this (Petrie *et al.* 1999, Piertney *et al.* 1999, Shorey *et al.* 2000). Various explanations for males rutting in the same location as relatives are possible in the study population, but little evidence exists to suggest which is the most likely. For example, selection may favour males rutting in the same location at the same time as their relatives because their male relatives may be more tolerant of them, and so the costs of competitive interactions between males could be reduced. Alternatively, males may return to rut in the same location as their fathers did because female relatives choose to mate with their male relatives for inclusive fitness benefits, and so males gain greater reproductive success. Finally, males may also choose to rut in their natal location because local information is available with respect to rutting behaviour: for example, males may have prior knowledge on how the acoustic properties of the landscape will affect the signal produced when roaring, or know where higher ground is to be gained to increase the probability of winning a fight.

7.3 Spatial autocorrelation

Finally, in chapter 6, I examine how the spatial genetic structure which results from female philopatry and the patterns of mating I have described affects our estimates of trait heritability. I show that environmental sources of similarity between individuals, when not accounted for, upwardly bias our estimates of heritability of home range size, birth weight and lifetime breeding success. Although bias in heritability estimates due to shared environment effects has been considered before now (Kruuk and Hadfield 2007), little work has been done to model such effects in wild populations more explicitly than by fitting nest box or maternal effects in animal models (but see Van der Jeugd and McCleery 2002).

I used two methods to account for shared environment effects in animal models. Firstly, I fitted autoregressive parameters to account for spatial autocorrelation in the traits considered. This method is well developed for forestry and agricultural trials, but has not been applied to animal models in wild animal populations. However, this method was not straightforward to undertake in terms of model convergence and parameter reliability. Further, whilst this method might prove very useful for accounting for spatial autocorrelation between nest boxes, it is probably not the most appropriate for species in which it is not easy to assign an animal one location per trait record. Secondly, I used a novel method, the multi-matrix method, in which I fitted a matrix of home range overlap, to animal models in addition to the pedigree. This method was relatively straightforward to apply, and the information which it incorporated was highly informative in explaining variance in the traits considered, despite its simplicity and limitations (see Chapter 6).

I therefore strongly suggest that the multi-matrix method should be more widely applied in quantitative genetics modelling of traits in this population, in order both to account for biases in estimates of genetic effects, but also to identify the strength of environmental effects. Separating genetic and environmental effects will be very important in understanding whether traits will respond to environmental change due to climate warming, and whether they are likely to do so by evolutionary change or phenotypic plasticity. Beyond this simple extension of my study, the scope for future research resulting from this chapter is wide. A previous study has suggested that there is sexual antagonism between male and female reproductive success in this red deer population (Foerster *et al.* 2007). However, my findings from chapter 5 suggest male and female relatives are likely to rut in similar locations; therefore shared environment effects (in this

case acting antagonistically) could generate this finding. For example, males may gain higher reproductive success by rutting in the most female dense areas, but effects of competition at those high densities might reduce female reproductive success (particularly as I have shown home range overlap explained approximately a quarter of the variance in female lifetime breeding success). Including autoregressive processes in models of genetic correlations may prove too difficult in a wild population where sample sizes are limited. However, fitting home range overlap only involves fitting another random effect to the model, and therefore should be more likely to converge.

In wild populations, the use of animal models is now widespread for estimating trait heritabilities, the genetic correlations underlying life history trade-offs and sexual antagonism, maternal effects, genotype by environment interactions, genotype by age interactions, selection and microevolution (reviewed in Kruuk *et al.* 2008). However, social and genetic structure is also ubiquitous in the majority of the types of populations in which these parameters are estimated (*e.g.* Coltman *et al.* 2003, Nussey *et al.* 2005, Foerster *et al.* 2006, see Storz 1999). As a consequence, environmental and genetic sources of similarity between individuals are likely to be confounded in such studies, potentially resulting in a source of bias in estimates of quantitative genetic parameters. Therefore, there is a fundamental need to account for such shared environment effects. The novel, yet relatively simple, method which I have presented to do this has important potential for our ability to use quantitative genetics in wild populations to examine questions of genetic variation, selection and evolution. Beyond quantitative genetic analysis, the multi-matrix method could also be used more widely to assess the variance explained in traits by causes of similarity between individuals. In particular, this technique may have exciting applications in the field of social network analysis, particularly in investigating whether association matrices between individuals explain any variance in fitness (Whitehead 2008, Wey *et al.* 2008). Multi-matrix techniques have already been used to incorporate dominance effects in the breeding literature (*e.g.* Boer and Arendonk 1992, Wei and Werf 1993). Ultimately, they may allow us to separate the role of genetics, interactions with other individuals and the environment in explaining variation in traits.

7.4 Limitations of the study

The long-term study of red deer on the North Block of the Isle of Rum has produced an incredible dataset on life-history traits, behaviour and genetic relationships in a wild

mammal. However, like all datasets, it is not without limitations, and here I will outline those relevant to this study.

Firstly, the sexual traits for which data exists are limited. For example, because the deer are not caught as adults, there are no annual measures of weight which would allow us to assess condition of males entering the rut. We also do not have long term data on the acoustic properties of roars. However, advances in technology, such as the possibility of using lasers to measure male leg length, or neck size, could potentially provide answers to this problem (Bergeron 2007).

Secondly, the spatial data that exists is taken from census records, and is correct only to the nearest 100m. Census data is limited in its utility because not all individuals are seen in any census, limiting the number of individuals for which home range size can be estimated. Further, censusing (at least the spring censuses) is necessarily limited to good weather conditions in which deer can be reliably recognised, which means censusing cannot reveal changes in spatial behaviour with respect to temporary changes in environmental conditions. Finally, because males emigrate from the population for the majority of the year, we have little information on their spatial movements, which limits our ability to understand environmental effects on male traits. More detailed information could be provided by radio-tracking individuals, although this also obviously comes with other problems, such as the loss of signal due to individuals displacing their tracking device on vegetation, the dangers of darting individuals to put in tracking devices, and statistical problems of radio tracking, such as deciding how often to collect data (Borger *et al.* 2006). However, the limitations to the spatial data available are unlikely to present significant difficulties for the analyses presented here. Figure 2.2 shows that on average females move between 200-500m between days, so the error around the 100m grid locations is reasonably trivial. Further, although individuals may move around the study area in response to weather conditions, they are likely to do so in the groups in which they normally associate, such that range sharing is likely to be unaffected by this.

Thirdly, an important limitation in all the analyses presented in this thesis is the pedigree data available. Whilst recent advances have substantially improved the red deer pedigree (Walling *et al.* 2010), it still contains missing links and in all likelihood, errors in paternity assignment. In categorical pedigrees, where assignments made above a particular threshold are accepted, the error surrounding pedigree assignments is ignored, potentially resulting in bias in parameter estimates (Devlin *et al.*1988, Neff *et al.* 2001, Nielsen *et al.*

2001, Hadfield *et al.* 2006). As an alternative, the use of fractional allocation has been suggested, in which a male is assigned a proportion of each offspring based on the probability of siring that offspring (Devlin *et al.* 1988, Neilsen *et al.* 2001). I address the problem of error in pedigree estimation in Appendix A, by using the iterations of the potential pedigree produced by the program MasterBayes rather than categorical assignments find no qualitative effect on the extent of re-mating, intra-lineage polygyny, co-ancestry or inbreeding observed. In general, most parameters are expected to be downwardly biased by errors in pedigree estimation (Pemberton 2008, Kruuk 2004, Keller *et al.* 2001, Charmantier and Réale 2005), including inbreeding (Pemberton 2008). However, the pedigree uncertainty is worth remembering when interpreting my findings, particularly when comparing the relative variance in a trait explained by genetic and environmental parameters. Further, any estimation of heritability which uses pedigree data to equate the strength of genetic relationships with the phenotypic covariance between pairs of individuals inevitably makes assumptions about the expected proportion of genes which are identical by descent in pairs of relatives. In the future, dense marker genotyping to will allow resolution of 'realised relatedness', the exact proportion of the genome which relatives have in common, to overcome this problem and to give estimates of heritability which are not confounded by environmental factors (Visscher *et al.* 2006).

Finally, the observational nature of the research presented in this thesis is a key limitation. Observing wild populations without interference from experimental procedures allows us to understand real world variability as well as limiting disturbance to the species studied. However, observational studies can generally only provide information on correlation, rather than causation. In particular, observational studies are limited by the difficulty of correlations between variables, such as the highly correlated measures of number of males rutting, number of immigrant males and rut sex ratio considered in Chapter 3. Further, without experimental controls, it is difficult to account for unmeasured variables that may be correlated with the measured data, potentially generating correlations between variables that are not directly related. Such variables make absolute confidence in knowing the true answer has been resolved almost impossible in an observational system, and as such we can only state that the final model is the best possible explanation of the phenomena observed given the data collected and current analytical abilities.

7.5 Understanding mating systems in the wild

As in this thesis, in recent years, the use of molecular data to assign paternities has greatly increased our understanding of the complexities of wild polygynous mating systems of many taxa. In pinnipeds, this has revealed evidence for females mating with the same male in multiple years (Amos *et al.* 1995); that variance in male reproductive success is lower than expected (Coltman *et al.* 2002); and that female choice may exist for unrelated and heterozygous males (Hoffman *et al.* 2007). In the horseshoe bat, authors have found evidence of re-mating and intra-lineage polygyny similar to that demonstrated in this thesis (Rossiter *et al.* 2005). Evidence has been found for sperm competition in Soay sheep (Preston *et al.* 2003). The role of extra-pair copulations in monogamous species has also been illuminated by molecular studies (reviewed in Hughes *et al.* 1998, Griffith *et al.* 2002), and several studies have combined molecular and simulation data to test whether animals avoid inbreeding (Keller *et al.* 1998, Hansson *et al.* 2007, Szulkin *et al.* 2009). Finally, a recent study has even used molecular data to reveal details of the mating system of field crickets in the wild (Rodríguez-Muñoz *et al.* 2010). Even where molecular data for paternity assignment is lacking, behavioural observations, particularly the use of spatial data, has revealed the potential for female choice in polygynous mammals (*e.g.* San Jose *et al.* 1998, Richard *et al.* 2008).

In a recent review, it has been suggested that the main unresolved problem currently facing sexual selection is whether female preferences for indirect benefits can evolve (Jones and Ratterman 2009). Testing the costs and benefits of female choice for indirect benefits in controlled conditions (generally, but not necessarily, in the lab) will obviously be important in resolving this problem. Ultimately though, there is a need to determine whether female choice actually occurs in species where males do not provide direct benefits in the wild, and to understand how important selection via female choice is relative to that from male-male competition. Disentangling female choice from male competition in the wild is not simple. However, I would argue that this thesis, and studies such as those mentioned above, indicate that we still have much to learn about the processes underlying sexual selection in the wild. Without doing so, we are unlikely to be able to produce reliable tests of the big theoretical questions of sexual selection in natural systems. For example, the assertion of Hoffman *et al.* (2007) that the lek paradox is resolved in fur seals by female choice for heterozygous and unrelated males is undermined in part by the problem that it is not clear that females in that population actually exert mate preferences (Kotiaho *et al.* 2008b). Therefore future studies should

pursue the use of combining spatial, molecular and simulation data to better understand the processes of sexual selection in wild systems.

7.6 Final thoughts

Sexual selection in wild populations is a complex and diverse thing. Male competition and female choice interact in unpredictable ways, selection varies temporally and spatially with environmental conditions, and selection can favour both plasticity and consistency in sexual traits. The analyses presented in this thesis highlight such intricacy in sexual selection in wild red deer and reveal surprising consequences for the genetic structure of the population. Understanding sexual selection reveals fundamental principles of nature: of selection, response to selection and the maintenance of variation within a population. Acquiring this understanding requires combining theoretical and laboratory studies with investigations of sexual selection in the wild, in order to identify the subtle interactions between behaviour, environment and genetic variation. The findings in this thesis should therefore underpin and inspire future work on the role of spatio-temporal environmental variation on sexual selection, as well as how sexual selection, inbreeding and kin selection are intertwined.

References

Albon, S. D., Staines, H.J., Guinness, F.E., Clutton-Brock, T.H. (1992) Density-dependent changes in the spacing behavior of female kin in red deer. *Journal of Animal Ecology,* **61,** 131-137.

Albon, S. D., Guinness, F. E. & Clutton-Brock, T. H. (1983) The influence of climatic variation on the birth weights of Red deer (Cervus elaphus). *Journal of Zoology,* **200,** 295-298.

Amos, B., Twiss, S., Pomeroy, P. & Anderson, S. (1995) Evidence for mate fidelity in the gray seal. *Science,* **268,** 1897-1899.

Amos, W., Wilmer, J. W., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. (2001) The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **268,** 2021-2027.

Andersson, M. (1994a) *Sexual Selection,* Princeton University Press, Princeton.

Andersson, S. (1994b) Costs of sexual advertising in the lekking Jackson's widowbird. *The Condor,* **96,** 1-10.

Apiolaza, L. A. (2006) ASReml cookbook. pp. http://uncronopio.org/ASReml/.

Arnold, S. J. & Duvall, D. (1994) Animal Mating Systems: A Synthesis Based on Selection Theory. *The American Naturalist,* **143,** 317-348.

Arnold, S. J. & Wade, M. J. (1984) On the Measurement of Natural and Sexual Selection: Theory. *Evolution,* **38,** 709-719.

Atkinson, B. (2008) kinship: mixed-effects Cox models, sparse matrices, and modeling data from large pedigrees. R package version 1.1.0-22.

Bartos, L., Bahbouh, R. & Vach, M. (2007) Repeatability of size and fluctuating asymmetry of antler characteristics in red deer (Cervus elaphus) during ontogeny. *Biological Journal of the Linnean Society,* **91,** 215-226.

Bateson, P. (1983) Optimal outbreeding. *Mate choice*. Cambridge University Press, Cambridge.

Berger, J. & Cunningham, C. (1991) Bellows, copulations, and sexual selection in bison (Bison bison). *Behavioral Ecology,* **2,** 1-6.

Berger, P. J., Negus, N. C. & Day, M. (1997) Recognition of Kin and Avoidance of Inbreeding in the Montane Vole, Microtus montanus. *Journal of Mammalogy,* **78,** 1182-1186.

Bergeron, P. (2007) Parallel Lasers for Remote Measurements of Morphological Traits. *The Journal of Wildlife Management,* **71,** 289-292.

Berglund, A. & Rosenqvist, G. (2001) Male pipefish prefer dominant over attractive females. *Behavioral Ecology,* **12,** 402-406.

Beyer, H. L. (2004) Hawth's analysis tools for ArcGIS. Available at <http://www.spatialecology.com/htools>

Bhattacharyya, A. (1943) On a measure of divergence between two statistical populations defined by their probability distributions. *Bulletin of the Calcutta Mathematical Society,* **35,** 99-109.

Birkhead, T. R. (2010) How stupid not to have thought of that: post-copulatory sexual selection. *Journal of Zoology,* **281,** 78-93.

Bjork, A. & Pitnick, S. (2006) Intensity of sexual selection along the anisogamy-isogamy continuum. *Nature,* **441,** 742-745.

Boer, I. J. M. & Arendonk, J. A. M. (1992) Prediction of additive and dominance effects in selected or unselected populations with inbreeding. *TAG Theoretical and Applied Genetics,* **84,** 451-459.

Boersma, P. & Weenink, D. (2010) Praat: doing phonetics by computer.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. & White, J.-S. S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution,* **24,** 127-135.

Bonenfant, C., Gaillard, J.-M., Klein, F. & Maillard, D. (2004) Variation in harem size of red deer (Cervus elaphus L.): the effects of adult sex ratio and age-structure. *Journal of Zoology,* **264,** 77-85.

Borger, L., Franconi, N., De Michele, G., Gantz, A., Meschi, F., Manica, A., Lovari, S. & Coulson, T. (2006) Effects of sampling regime on the mean and variance of home range size estimates. *Journal of Animal Ecology,* **75,** 1393-1405.

Borries, C., Launhardt, K., Epplen, C., Epplen, J. T. & Winkler, P. (1999) Males as infant protectors in Hanuman langurs (Presbytis entellus) living in multimale groups - defence pattern, paternity and sexual behaviour. *Behavioral Ecology and Sociobiology,* **46,** 350-356.

Botero, C. A., Pen, I., Komdeur, J. & Weissing, F. J. (2010) The evolution of individual variation in communication strategies. *Evolution,* **64,** 3123-3133.

Botero, C. A., Rossman, R. J., Caro, L. M., Stenzler, L. M., Lovette, I. J., de Kort, S. R. & Vehrencamp, S. L. (2009) Syllable type consistency is related to age, social status and reproductive success in the tropical mockingbird. *Animal Behaviour,* **77,** 701-706.

Both, C. & Visser, M. E. (2001) Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature,* **411,** 296-298.

Bradbury, J. W. & Vehrencamp, S. L. (2000) Economic models of animal communication. *Animal Behaviour,* **59,** 259-268.

Brommer, J. E., Merilä, J., Sheldon, B. C. & Gustafsson, L. (2005) Natural selection and genetic variation for reproductive reaction norms in a wild bird population. *Evolution,* **59,** 1362-1371.

Brooks, R. & Kemp, D. J. (2001) Can older males deliver the good genes? *Trends in Ecology & Evolution,* **16,** 308-313.

Buchan, J. C., Alberts, S. C., Silk, J. B. & Altmann, J. (2003) True paternal care in a multi-male primate society. *Nature,* **425,** 179-181.

Burgueno, J. C., A., Crossa, J. Banziger, M. Gilmour, A.R., Cullis, B.R. (2000) *User's guide for spatial analysis of field variety trials using ASREML,* CIMMYT, Mexico.

Bussière, L., Hunt, J., Stölting, K., Jennions, M. & Brooks, R. (2008) Mate choice for genetic quality when environments vary: suggestions for empirical progress. *Genetica,* **134,** 69-78.

Byers, B. E. (2007) Extrapair paternity in chestnut-sided warblers is correlated with consistent vocal performance. *Behavioral Ecology,* **18,** 130-136.

Byers, J. A., Moodie, J. D. & Hall, N. (1994) Pronghorn females choose vigorous mates. *Animal Behaviour,* **47,** 33-43.

Byers, J. A., Wiseman, P. A., Jones, L. & Roffe, T. J. (2005) A large cost of female mate sampling in pronghorn. *The American Naturalist,* **166,** 661-668.

Calenge, C. (2006) The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling,* **197,** 516-519.

Cam, E., Monnat J, J.-Y. & Royle, A. (2004) Dispersal and individual quality in a long lived species. *Oikos,* **106,** 386-398.

Candolin, U. (1999) Male-male competition facilitates female choice in sticklebacks. *Proceedings: Biological Sciences,* **266,** 785-789.

Candolin, U. (2003) The use of multiple cues in mate choice. *Biological Reviews,* **78,** 575-595.

Carbone, C. & Taborsky, M. (1996) Mate choice or harassment avoidance? A question of female control at the lek. *Behavioral Ecology,* **7,** 370-373.

Carranza, J. (2009) Defining sexual selection as sex-dependent selection. *Animal Behaviour,* **77,** 3.

Carranza, J. (1995) Female attraction by males versus sites in territorial rutting red deer. *Animal Behaviour,* **50,** 445-453.

Carranza, J. & Valencia, J. (1999) Red deer females collect on male clumps at mating areas. *Behavioral Ecology,* **10,** 525-532.

Cézilly, F., Dubois, F. & Pagel, M. (2000) Is mate fidelity related to site fidelity? A comparative analysis in Ciconiiforms. *Animal Behaviour,* **59,** 1143-1152.

Chaine, A. S. & Lyon, B. E. (2008) Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the Lark Bunting. *Science,* **319,** 459-462.

Chapman, B., Morrell, L. & Krause, J. (2009) Plasticity in male courtship behaviour as a function of light intensity in guppies. *Behavioral Ecology and Sociobiology,* **63,** 1757-1763.

Charlesworth, B. (1987) The heritability of fitness. In *Sexual selection: testing the ideas* (eds J. W. Bradbury & M. B. Andersson), pp. 21-40. Wiley, Chichester, UK.

Charlesworth, B. & Charlesworth, D. (1999) The genetic basis of inbreeding depression. *Genetical Research,* **74**.

Charlton, B. D., Huang, Y. & Swaisgood, R. R. (2009) Vocal discrimination of potential mates by female giant pandas (Ailuropoda melanoleuca). *Biology Letters,* **5,** 597-599.

Charlton, B. D., McComb, K. & Reby, D. (2008) Free-ranging red deer hinds show greater attentiveness to roars with formant frequencies typical of young males. *Ethology,* **114,** 1023-1031.

Charlton, B. D., Reby, D. & McComb, K. (2007a) Female red deer prefer the roars of larger males. *Biology Letters,* **3,** 382-385.

Charlton, B. D., Reby, D. & McComb, K. (2007b) Female perception of size-related formant shifts in red deer, Cervus elaphus. *Animal Behaviour,* **74,** 707-714.

Charmantier, A. & Garant, D. (2005) Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society B: Biological Sciences,* **272,** 1415-1425.

Charmantier, A., Kruuk, L. E. B., Blondel, J. & Lambrechts, M. M. (2004) Testing for microevolution in body size in three blue tit populations. *Journal of Evolutionary Biology,* **17,** 732-743.

Charmantier, A. & Réale, D. (2005) How do misassigned paternities affect the estimation of heritability in the wild? *Molecular Ecology,* **14,** 2839-2850.

Chesser, R. K. (1991) Gene Diversity and Female Philopatry. *Genetics,* **127,** 437-447.

Choudhury, S. (1995) Divorce in birds: a review of the hypotheses. *Animal Behaviour,* **50,** 413-429.

Clements, M., Clutton-Brock, T., Albon, S., Pemberton, J. & Kruuk, L. (2010) Getting the timing right: antler growth phenology and sexual selection in a wild red deer population. *Oecologia,* **164,** 357-368.

Clements, M. N., Clutton-Brock, T. H., Guinness, F. E., Pemberton, J. M. & Kruuk, L. E. B. (2010) Variances and covariances of phenological traits in a wild mammal population. *Evolution,* **65,** 788-801.

Cliff, A. & Ord, J. K. (1981) *Spatial Processes,* Pion, London.

Clutton-Brock, T. & Albon, S. D. (1979) The Roaring of Red Deer and the Evolution of Honest Advertisement. *Behaviour,* **69,** 145-170.

Clutton-Brock, T. & McAuliffe, K. (2009) Female mate choice in mammals. *The Quarterly Review of Biology,* **84,** 3-27.

Clutton-Brock, T. & McComb, K. (1993) Experimental tests of copying and mate choice in fallow deer (Dama dama). *Behavioral Ecology,* **4,** 191-193.

Clutton-Brock, T. H. (1989) Review Lecture: Mammalian Mating Systems. *Proceedings of the Royal Society of London. B. Biological Sciences,* **236,** 339-372.

Clutton-Brock, T. H., Albon, S. D., Gibson, R. M. & Guinness, F. E. (1979) The logical stag: Adaptive aspects of fighting in red deer (Cervus elaphus L.). *Animal Behaviour,* **27,** 211-225.

Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. (1982) Competition between female relatives in a matrilocal mammal. *Nature,* **300,** 178-180.

Clutton-Brock, T. H., Coulson, T. N., Milner-Gulland, E. J., Thomson, D. & Armstrong, H. M. (2002) Sex differences in emigration and mortality affect optimal management of deer populations. *Nature,* **415,** 633-637.

Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. (1982) *Red deer: Behaviour and ecology of two sexes.,* Edinburgh University Press, Edinburgh.

Clutton-Brock, T. H. & Harvey, P. H. (1978) Mammals, resources and reproductive strategies. *Nature,* **273,** 191-195.

Clutton-Brock, T. H. & Parker, G. A. (1992) Potential reproductive rates and the operation of sexual selection. *The Quarterly Review of Biology,* **67,** 437-456.

Clutton-Brock, T. H., Price, O. F. & MacColl, A. D. C. (1992) Mate retention, harassment, and the evolution of ungulate leks. *Behavioral Ecology,* **3,** 234-242.

Clutton-Brock, T. H., Rose, K. E. & Guinness, F. E. (1997) Density related changes in sexual selection in red deer. *Proceedings of the Royal Society B: Biological Sciences,* **264,** 1509-1516.

Cockburn, A., Osmond, H. L. & Double, M. C. (2008) Swingin' in the rain: condition dependence and sexual selection in a capricious world. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 605-612.

Coltman, D. W., Bancroft, D. R., Robertson, A., Smith, J. A., Clutton-Brock, T. H. & Pemberton, J. M. (1999) Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Molecular Ecology,* **8,** 1199-1209.

Coltman, D. W., Bowen, W. D. & Wright, J. M. (1998) Male mating success in an aquatically mating pinniped, the harbour seal (Phoca vitulina), assessed by microsatellite DNA markers. *Molecular Ecology,* **7,** 627-638.

Coltman, D. W., Pilkington, J. G. & Pemberton, J. M. (2003) Fine-scale genetic structure in a free-living ungulate population. *Molecular Ecology,* **12,** 733-742.

Coltman, D. W., Smith, J. A., Bancroft, D. R., Pilkington, J., MacColl, A. D. C., Clutton-Brock, T. H. & Pemberton, J. M. (1999) Density Dependent Variation in Lifetime Breeding Success and Natural and Sexual Selection in Soay Rams. *The American Naturalist,* **154,** 730-746.

Conradt, L., Clutton-Brock, T. H. & Guinness, F. E. (1999) The relationship between habitat choice and lifetime reproductive success in female red deer. *Oecologia,* **120,** 218-224.

Cordero, C. (1996) On the evolutionary origin of nuptial seminal gifts in insects. *Journal of Insect Behavior,* **9,** 969-974.

Cornwallis, C. K. & Uller, T. (2010) Towards an evolutionary ecology of sexual traits. *Trends in Ecology & Evolution,* **25,** 145-152.

Costa e Silva, J., Dutkowski, G. W. & Gilmour, A. R. (2001) Analysis of early tree height in forest genetic trials is enhanced by including a spatially correlated residual. *Canadian Journal of Forest Research,* **31,** 1887-1893.

Coster, A. (2008) pedigree: Pedigree functions. R package version 1.1.

Coulson, T., Albon, S., Guinness, F., Pemberton, J. & Clutton-Brock, T. (1997) Population substructure, local density, and calf winter survival in red deer (*Cervus elaphus*). *Ecology,* **78,** 852-863.

Coulson, T., Albon, S., Jon, S. & Pemberton, J. (1999) Microsatellite Loci Reveal Sex-Dependent Responses to Inbreeding and Outbreeding in Red Deer Calves. *Evolution,* **53,** 1951-1960.

Coulson, T., Guinness, F., Pemberton, J. & Clutton-Brock, T. (2004) The Demographic Consequences of Releasing a Population of Red Deer from Culling. *Ecology,* **85,** 411-422.

Coulson, T., Kruuk, L. E. B., Tavecchia, G., Pemberton, J. M. & Clutton-Brock, T. H. (2003) Estimating selection on neonatal traits in red deer using elasticity path analysis. *Evolution,* **57,** 2879-2892.

Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. (1998) Microsatellites Reveal Heterosis in Red Deer. *Proceedings: Biological Sciences,* **265,** 489-495.

Cox, C. R. & Boeuf, B. J. L. (1977) Female Incitation of Male Competition: A Mechanism in Sexual Selection. *The American Naturalist,* **111,** 317-335.

Crawley, M. J. (2007) *The R Book,* John Wiley & Sons Ltd, Chichester.

Crick, H. Q. P. & Sparks, T. H. (1999) Climate change related to egg-laying trends. *Nature,* **399,** 423-423.

Croft, D., James, R., Thomas, P., Hathaway, C., Mawdsley, D., Laland, K. & Krause, J. (2006) Social structure and co-operative interactions in a wild population of guppies (Poecilia reticulata). *Behavioral Ecology and Sociobiology,* **59,** 644-650.

Croft, D. P., Krause, J. & James, R. (2004) Social networks in the guppy (Poecilia reticulata). *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **271,** S516-S519.

Crow, J. F. (1958) Some possibilites for measuring selection intensities in man. *Human Biology,* **30,** 1-13.

Cullis, B. R. & Gleeson, A. C. (1989) Efficiency of Neighbor Analysis for Replicated Variety Trials in Australia. *Journal of Agricultural Science,* **113,** 233-239.

Cullis, B. R. & Gleeson, A. C. (1991) Spatial-Analysis of Field Experiments - an Extension to 2 Dimensions. *Biometrics,* **47,** 1449-1460.

Dahle, B. & Swenson, J. E. (2003) Seasonal range size in relation to reproductive strategies in brown bears Ursus arctos. *Journal of Animal Ecology,* **72,** 660-667.

Daniels, M. & McClean, C. (2003) Red deer calf tagging programmes in Scotland — an analysis *Deer (The Journal of the British Deer Society),* **12,** 420–423.

Danielson-François, A. M., Kelly, J. K. & Greenfield, M. D. (2006) Genotype × environment interaction for male attractiveness in an acoustic moth: evidence for plasticity and canalization. *Journal of Evolutionary Biology,* **19,** 532-542.

Darwin, C. (1871) *The Descent of Man, and Selection in Relation to Sex (1st ed.),* John Murray, London.

De Solla, S. R., Bonduriansky, R. & Brooks, R. J. (1999) Eliminating autocorrelation reduces biological relevance of home range estimates. *Journal of Animal Ecology,* **68,** 221-234.

Devlin, B., Roeder, K. & Ellstrand, N. C. (1988) Fractional paternity assignment: theoretical development and comparison to other methods. *TAG Theoretical and Applied Genetics,* **76,** 369-380.

Dingemanse, N. J., Kazem, A. J. N., Réale, D. & Wright, J. (2009) Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution,* **25,** 81-89.

Double, M. C., Peakall, R., Beck, N. R. & Cockburn, A. (2005) Dispersal, Philopatry, and Infidelity: Dissecting Local Genetic Structure in Superb Fairy-Wrens (Malurus cyaneus). *Evolution,* **59,** 625-635.

Doutrelant, C. & McGregor, P. K. (2000) Eavesdropping and mate choice in female fighting fish. *Behaviour,* **137,** 1655-1669.

Dreiss, A. N., Cote, J., Richard, M., Federici, P. & Clobert, J. Age- and sex-specific response to population density and sex ratio. *Behavioral Ecology,* **21,** 356-364.

Dubois, F. & Cézilly, F. (2002) Breeding success and mate retention in birds: a meta-analysis. *Behavioral Ecology and Sociobiology,* **52,** 357-364.

Duraes, R., Loiselle, B. A. & Blake, J. G. (2007) Intersexual spatial relationships in a lekking species: blue-crowned manakins and female hot spots. *Behavioral Ecology,* **18,** 1029-1039.

Dutkowski, G. W., Silva, J. C. E., Gilmour, A. R. & Lopez, G. A. (2002) Spatial analysis methods for forest genetic trials. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* **32,** 2201-2214.

Ellegren, H. & Sheldon, B. C. (2008) Genetic basis of fitness differences in natural populations. *Nature,* **452,** 169-175.

Ellner, S. & Hairston Jr., N. G. (1994) Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *The American Naturalist,* **143,** 403-417.

Elowson, A. M. & Snowdon, C. T. (1994) Pygmy marmosets, Cebuella pygmaea, modify vocal structure in response to changed social environment. *Animal Behaviour,* **47,** 1267-1277.

Emlen, S. T. & Oring, L. W. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science,* **197,** 215-223.

Endler, J. (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes,* **9,** 173-190.

Endler, J. (1986) *Natural selection in the wild,* Princeton University Press.

Endler, J. & Rojas, B. (2009) The Spatial Pattern of Natural Selection When Selection Depends on Experience. *The American Naturalist,* **173,** E62-E78.

Eshel, I. (1979) Sexual selection, population density, and availability of mates. *Theoretical Population Biology,* **16,** 301-314.

Fan,Y., Morgan, B.J.T., Catchpole, E.A. and Coulson, T.N. (2003) Modelling the survival and dispersal of red deer using mark-recapture-recovery data. *Report UKC/IMS/03/19,* . IMS University of Kent, Canterbury, UK.

Falconer, D. S. (1989) *Introduction to quantitative genetics,* Longman, London and New York.

Fieberg, J. & Kochanny, C. O. (2005) Quantifying home-range overlap: The importance of the utilization distribution. *Journal of Wildlife Management,* **69,** 1346-1359.

Fisher, D. O. & Lara, M. C. (1999) Effects of body size and home range on access to mates and paternity in male bridled nailtail wallabies. *Animal Behaviour,* **58,** 121-130.

Fitch, W. H. & Hauser, M. D. (2003) Unpacking "Honesty": vertebrate vocal production and the evolution of acoustic signals. (eds A. M. Simmons, R. R. Fay & A. N. Popper), pp. 65-137. Springer New York, New York.

Fitch, W. T. (1997) Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. *The Journal of the Acoustical Society of America,* **101,** 3136.

Fitze, P. S. & Le Galliard, J.-F. (2008) Operational sex ratio, sexual conflict and the intensity of sexual selection. *Ecology Letters,* **11,** 432-439.

Fleming, I. A. & Gross, M. R. (1994) Breeding Competition in a Pacific Salmon (Coho: Oncorhynchus kisutch): Measures of Natural and Sexual Selection. *Evolution,* **48,** 637-657.

Foerster, K., Coulson, T., Sheldon, B. C., Pemberton, J. M., Clutton-Brock, T. H. & Kruuk, L. E. B. (2007) Sexually antagonistic genetic variation for fitness in red deer. *Nature,* **447,** 1107-1110.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T. & Kempenaers, B. (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature,* **425,** 714-717.

Foerster, K., Valcu, M., Johnsen, A. & Kempenaers, B. (2006) A spatial genetic structure and effects of relatedness on mate choice in a wild bird population. *Molecular Ecology,* **15,** 4555-4567.

Forstmeier, W. (2004) Female resistance to male seduction in zebra finches. *Animal Behaviour,* **68,** 1005-1015.

Forstmeier, W. (2007) Do Individual Females Differ Intrinsically in Their Propensity to Engage in Extra-Pair Copulations? *PLoS ONE,* **2,** e952.

Forstmeier, W. & Birkhead, T. R. (2004) Repeatability of mate choice in the zebra finch: consistency within and between females. *Animal Behaviour,* **68,** 1017-1028.

Fortin, M. D., M.R.T. (2005) *Spatial analysis: a guide for ecologists*, Cambridge University Press.

Fortin, M. J. & Dale, M. R. T. (2009) Spatial Autocorrelation in Ecological Studies: A Legacy of Solutions and Myths. *Geographical Analysis,* **41,** 392-397.

Frere, C. H., Krutzen, M., Mann, J., Connor, R. C., Bejder, L. & Sherwin, W. B. (2010) Social and genetic interactions drive fitness variation in a free-living dolphin population. *Proceedings of the National Academy of Sciences of the United States of America,* **107,** 19949-19954.

Galeotti, P., Saino, N., Sacchi, R. & Møller, A. P. (1997) Song correlates with social context, testosterone and body condition in male barn swallows. *Animal Behaviour,* **53,** 687-700.

Garant, D., Kruuk, L. E. B., Wilkin, T. A., McCleery, R. H. & Sheldon, B. C. (2005) Evolution driven by differential dispersal within a wild bird population. *Nature,* **433,** 60-65.

Garant, D., Sheldon, B. C. & Gustafsson, L. (2004) Climatic and temporal effects on the expression of secondary sexual characters: Genetic and environmental components. *Evolution,* **58,** 634-644.

Geist, V. (1982) Adaptive behavioural strategies. *Elk of North America: ecology and management* (eds J. W. Thomas & D. E. Toweill), pp. 219-277. Stackpole Books, Harrisburg.

Gerhardt, H. C. (1994) The evolution of vocalization in frogs and toads. *Annual Review of Ecology and Systematics,* **25,** 293-324.

Gibson, R. M. & Guinness, F. E. (1980) Differential reproduction among red deer (Cervus-Elaphus) stags on Rhum. *Journal of Animal Ecology,* **49,** 199-208.

Gienapp, P., Teplitsky, C., Alho, J. S., Mills, J. A. & Merilä, J. (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology,* **17,** 167-178.

Giesselmann, U. C., Wiegand, T., Meyer, J., Vogel, M. & Brandl, R. (2008) Spatial distribution of communal nests in a colonial breeding bird: benefits without costs? *Austral Ecology,* **33,** 607-613.

Gillespie, J. H. & Turelli, M. (1989) Genotype-environment interactions and the maintenance of polygenic variation. *Genetics,* **121,** 129-138.

Gilmour, A. R., Cullis B.R. & Verbyla, A.P. (1997) Accounting for natural extraneous variaion in the analysis of field experiments. *JABES,* **2,** 269-273.

Gilmour, A. R., Gogel, B. J., Cullis, B. R. & Thompson, R. (2009) *ASReml User Guide,* VSN International Ltd., Hemel Hempstead, UK.

Godin, J. G. & Dugatkin, L. A. (1996) Female mating preference for bold males in the guppy, Poecilia reticulata. *Proceedings of the National Academy of Sciences of the United States of America,* **93,** 10262-10267.

Godin, J. G. J. (1995) Predation risk and alternative mating tactics in male Trinidadian guppies (*Poecilia reticulata*). *Oecologia,* **103,** 224-229.

Gosden, T. P. & Svensson, E. I. (2008) Spatial and temproal dynamics in a sexual selection mosaic. *Evolution,* **62,** 845-856.

Grafen, A. (1987) Measuring sexual selection: why bother? *Sexual selection: testing the alternatives* (eds J. W. Bradbury & M. Andersson), pp. 221-233. John Wiley & Sons.

Grant, B. R. (1985) Selection on Bill Characters in a Population of Darwin's Finches: Geospiza conirostris on Isla Genovesa, Galapagos. *Evolution,* **39,** 523-532.

Grant, B. R. & Grant, P. R. (1987) Mate choice in Darwin's Finches. *Biological Journal of the Linnean Society,* **32,** 247-270.

Grant, B. R. & Grant, P. R. (1989) Natural Selection in a Population of Darwin's Finches. *The American Naturalist,* **133,** 377-393.

Greenfield, M. D. & Rodriguez, R. L. (2004) Genotype-environment interaction and the reliability of mating signals. *Animal Behaviour,* **68,** 1461-1468.

Griffin, A. S. & West, S. A. (2003) Kin Discrimination and the Benefit of Helping in Cooperatively Breeding Vertebrates. *Science,* **302,** 634-636.

Griffith, S. C., Owens, I. P. F. & Burke, T. (1999) Environmental determination of a sexually selected trait. *Nature,* **400,** 358-360.

Griffith, S. C., Owens, I. P. F. & Thuman, K. A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology,* **11,** 2195-2212.

Griffith, S. C. & Sheldon, B. C. (2001) Phenotypic plasticity in the expression of sexually selected traits: neglected components of variation. *Animal Behaviour,* **61,** 987-993.

Guinness, F., Lincoln, G. A. & Short, R. V. (1971) The reproductive cycle of the female red deer, *Cervus elaphus L. Journal of Reproduction and Fertility* **27,** 427-438.

Guinness, F. E., Clutton-Brock, T. H. & Albon, S. D. (1978) Factors affecting calf mortality in red deer (*Cervus elaphus)*. *Journal of Animal Ecology,* **47,** 817-832.

Hadfield, J. D., Richardson, D. S. & Burke, T. (2006) Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. *Molecular Ecology,* **15,** 3715-3730.

Hadfield, J. D., Wilson, A. J., Garant, D., Sheldon, B. C. & Kruuk, L. E. B. (2010) The misuse of BLUP in ecology and evolution. *The American Naturalist,* **175,** 116-125.

Haining, R. (2004) *Spatial data analysis: theory and practice,* Cambridge University Press.

Hamilton, W. D. (1963) The evolution of altruistic behavior. *The American Naturalist,* **97,** 354-356.

Hansen, T. F. & Price, D. K. (1995) Good genes and old age: Do old mates provide superior genes? *Journal of Evolutionary Biology,* **8,** 759-778.

Hansson, B., Jack, L., Christians, J. K., Pemberton, J. M., Ãkesson, M., Westerdahl, H., Bensch, S. & Hasselquist, D. (2007) No evidence for inbreeding avoidance in a great reed warbler population. *Behavioral Ecology,* **18,** 157-164.

Hansson, B. & Westerberg, L. (2002) On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology,* **11,** 2467-2474.

Harris, T. R., Fitch, W. T., Goldstein, L. M. & Fashing, P. J. (2006) Black and white colobus monkey (Colobus guereza) roars as a source of both honest and exaggerated information about body mass. *Ethology,* **112,** 911-920.

Heinsohn, R., Dunn, P., Legge, S. & Double, M. (2000) Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs. *Proceedings of the Royal Society B: Biological Sciences,* **267,** 243-249.

Hirotani, A. (1994) Dominance rank, copulatory-behavior and estimated reproductive success in male reindeer. *Animal Behaviour,* **48,** 929-936.

Hoffman, J. I., Forcada, J., Trathan, P. N. & Amos, W. (2007) Female fur seals show active choice for males that are heterozygous and unrelated. *Nature,* **445,** 912-914.

Hoffmann, A. A. & Merilä, J. (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, **14,** 96-101.

Hogg, J. T. & Forbes, S. H. (1997) Mating in bighorn sheep: frequent male reproduction via a high-risk "unconventional" tactic. *Behavioral Ecology and Sociobiology,* **41,** 33-48.

Hoglund, J., Alatalo, R. V., Lundberg, A., Rintamaki, P. T. & Lindell, J. (1999) Microsatellite markers reveal the potential for kin selection on black grouse leks. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **266,** 813-816.

Höglund, J. & Shorey, L. (2003) Local genetic structure in a white-bearded manakin population. *Molecular Ecology,* **12,** 2457-2463.

Holand, Ø., Askim, K. R., Røed, K. H., Weladji, R. B., Gjøstein, H. & Nieminen, M. (2007) No evidence of inbreeding avoidance in a polygynous ungulate: the reindeer (Rangifer tarandus). *Biology Letters,* **3,** 36-39.

Hollén, L. I. & Manser, M. B. (2007) Motivation before meaning: Motivational information encoded in meerkat alarm calls develops earlier than referential information. *The American Naturalist,* **169,** 758-767.

Houle, D. (1992) Comparing Evolvability and Variability of Quantitative Traits. *Genetics,* **130,** 195-204.

Howard, R. D., Moorman, R. S. & Whiteman, H. H. (1997) Differential effects of mate competition and mate choice on eastern tiger salamanders. *Animal Behaviour,* **53,** 1345-1356.

Huck, M., Davison, J. & Roper, T. J. (2008) Comparison of two sampling protocols and four home-range estimators using radio-tracking data from urban badgers Meles meles. *Wildlife Biology,* **14,** 467-477.

Hughes, C. (1998) Integrating molecular techniques with field methods in studies of social behavior: A revolution results. *Ecology,* **79,** 383-399.

Hulbert, S. H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs,* **54,** 187-211.

Hunt, J., Breuker, C. J., Sadowski, J. A. & Moore, A. J. (2009) Male–male competition, female mate choice and their interaction: determining total sexual selection. *Journal of Evolutionary Biology,* **22,** 13-26.

Iason, G. R. & Guinness, F. E. (1985) Synchrony of oestrus and conception in red deer (Cervus elaphus L.). *Animal Behaviour,* **33,** 1169-1174.

Ims, R. (1988) The potential for sexual selection in males: Effect of sex ratio and spatiotemporal distribution of receptive females. *Evolutionary Ecology,* **2,** 338-352.

Ingleby, F. C., Hunt, J. & Hosken, D. J. (2010) The role of genotype-by-environment interactions in sexual selection. *Journal of Evolutionary Biology,* **23,** 2031-2045.

Isaac, J. L. (2009) Effects of climate change on life history: implications for extinction risk in mammals. *Endagered species research,* **7,** 115-123.

Järvenpää, M. & Lindström, K. (2004) Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby Pomatoschistus minutus. *Proceedings of the Royal Society of London B: Biological Sciences,* **271,** 2361-2365.

Jason, B. W., Brodie, E. D. & Moore, A. J. (1999) Interacting Phenotypes and the Evolutionary Process. II. Selection Resulting from Social Interactions. *The American Naturalist,* **153,** 254-266.

Jennions, M. D. & Petrie, M. (1997) Variation in mate choice and mating preferences: A review of causes and consequences. *Biological Reviews of the Cambridge Philosophical Society,* **72,** 283-327.

Jirotkul, M. (1999) Population density influences male-male competition in guppies. *Animal Behaviour,* **58,** 1169-1175.

Jirotkul, M. (2000) Operational sex ratio influences the opportunity for sexual selection in guppies. *Journal of Fish Biology,* **56,** 739-741.

Johnstone, R. A. (1995) Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biological Reviews,* **70,** 1-65.

Jones, A. G. (2009) On the opportunity for sexual selection, the Bateman Gradient and the maximum intensity of sexual selection. *Evolution,* **63,** 1673-1684.

Jones, A. G., Arguello, J. R. & Arnold, S. J. (2004) Molecular parentage analysis in experimental newt populations: the response of mating system measures to variation in the operational sex ratio. *The American Naturalist,* **164,** 444-456.

Jones, A. G. & Ratterman, N. L. (2009) Mate choice and sexual selection: What have we learned since Darwin? *Proceedings of the National Academy of Sciences,* **106,** 10001-10008.

Kangas, N. & Lindström, K. (2001) Male interactions and female mate choice in the sand goby, Pomatoschistus minutus. *Animal Behaviour,* **61,** 425-430.

Kasumovic, M. M., Bruce, M. J., Andrade, M. C. B. & Herberstein, M. E. (2008) Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. *Evolution,* **62,** 2316-2325.

Keller, L. F. & Arcese, P. (1998) No evidence for inbreeding avoidance in a natural population of song sparrows (Melospiza melodia). *The American Naturalist,* **152,** 380-392.

Keller, L. F., Grant, P. R., Grant, B. R. & Petren, K. (2001) Heritability of morphological traits in Darwin/'s Finches: misidentified paternity and maternal effects. *Heredity,* **87,** 325-336.

Keller, L. F. & Waller, D. M. (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution,* **17,** 230-241.

Kelly, C. D. (2008) Identifying a causal agent of sexual selection on weaponry in an insect. *Behavioral Ecology,* **19,** 184-192.

Kempenaers, B. (1993) The Use of a Breeding Synchrony Index. *Ornis Scandinavica,* **24,** 84.

Kent, C., Azanchi, R., Smith, B., Formosa, A. & Levine, J. D. (2008) Social context influences chemical communication in D. melanogaster males. *Current Biology,* **18,** 1384-1389.

Kenward, R. E., Clarke, R. T., Hodder, K. H. & Walls, S. S. (2001) Density and linkage estimators of home range: Nearest-neighbor clustering defines multinuclear cores. *Ecology,* **82,** 1905-1920.

Kernohan, B. J., Gitzen, R. A. & Millspaugh, J. J. (2001) Anaysis of animal space use and movements. *Radio tracking animal populations* (eds J. J. Millspaugh & J. M. Marzluff). Academic Press, San Diego, California.

Kissling, W. D. & Carl, G. (2008) Spatial autocorrelation and the selection of simultaneous autoregressive models. *Global Ecology and Biogeography,* **17,** 59-71.

Klug, H., Heuschele, J., Jennions, M. D. & Kokko, H. (2010a) The mismeasurement of sexual selection. *Journal of Evolutionary Biology,* **23,** 447-462.

Klug, H., Lindström, K. & Kokko, H. (2010b) Who to include in measures of sexual selection is no trivial matter. *Ecology Letters,* **13,** 1094-1102.

Knell, R. J. (2009) Population density and the evolution of male aggression. *Journal of Zoology,* **278,** 83-90.

Koenig, W. D. & Albano, S. S. (1986) On the measurement of sexual selection. *The American Naturalist,* **127,** 403-409.

Kokko, H., Brooks, R., Jennions, M. D. & Morley, J. (2003) The evolution of mate choice and mating biases. *Proceedings of the Royal Society B: Biological Sciences,* **270,** 653-664.

Kokko, H. & Heubel, K. (2008) Condition-dependence, genotype-by-environment interactions and the lek paradox. *Genetica,* **132,** 209-216.

Kokko, H. & Johnstone, R. A. (2002) Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* **357,** 319-330.

Kokko, H., Mackenzie, A., Reynolds, J. D., Lindstrøm, J. & Sutherland, W. J. (1999) Measures of Inequality Are Not Equal. *The American Naturalist,* **154,** 358-382.

Kokko, H. & Monaghan, P. (2001) Predicting the direction of sexual selection. *Ecology Letters,* **4,** 159-165.

Kokko, H. & Ots, I. (2006) When not to avoid inbreeding. *Evolution,* **60,** 467-475.

Kokko, H. & Rankin, D. J. (2006) Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **361,** 319-334.

Komers, P. E., Messier, F. & Gates, C. C. (1994) Plasticity of reproductive-behaviour in wood bison bulls- when subadults are given a chance. *Ethology, ecology and evolution,* **6,** 313-330.

Kontiainen, P., Pietiøinen, H., Huttunen, K., Karell, P., Kolunen, H. & Brommer, J. E. (2009) Aggressive Ural owl mothers recruit more offspring. *Behavioral Ecology,* **20,** 789-796.

Kotiaho, J. S., Le Bas, N. R., Puurtinen, M. & Tomkins, J. L. (2008a) On the resolution of the lek paradox. *Trends in Ecology & Evolution,* **23,** 1-3.

Kotiaho, J. S., LeBas, N. R., Puurtinen, M. & Tomkins, J. L. (2008b) On female choice, heterozygosity and the lek paradox. . *Animal Behaviour,* **75,** e1:e3.

Kruuk, L. E. B. (2004) Estimating genetic parameters in natural populations using the 'animal model'. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* **359,** 873-890.

Kruuk, L. E. B., Clutton-Brock, T. H., Slate, J., Pemberton, J. M., Brotherstone, S. & Guinness, F. E. (2000) Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences of the United States of America,* **97,** 698-703.

Kruuk, L. E. B. & Hadfield, J. D. (2007) How to separate genetic and environmental causes of similarity between relatives. *Journal of Evolutionary Biology,* **20,** 1890-1903.

Kruuk, L. E. B. & Hill, W. G. (2008) Introduction. Evolutionary dynamics of wild populations: the use of long-term pedigree data. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 593-596.

Kruuk, L. E. B., Sheldon, B. C. & Merila, J. (2002a) Severe inbreeding depression in collared flycatchers (Ficedula albicollis). *Proceedings of the Royal Society B: Biological Sciences,* **269,** 9.

Kruuk, L. E. B., Slate, J., Pemberton, J. M., Brotherstone, S., Guinness, F. & Clutton-Brock, T. (2002b) Antler size in red deer: heritability and selection but no evolution. *Evolution,* **56,** 1683-1695.

Kruuk, L. E. B., Slate, J. & Wilson, A. J. (2008) New Answers for Old Questions: The Evolutionary Quantitative Genetics of Wild Animal Populations. *Annual Review of Ecology, Evolution, and Systematics,* **39,** 525-548.

Kvarnemo, C. & Ahnesjo, I. (1996) The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution,* **11,** 404-408.

Labisky, R. F. & Fritzen, D. E. (1998) Spatial mobility of breeding female white-tailed deer in a low-density population. *Journal of Wildlife Management,* **62,** 1329-1334.

Lambin, X. & Krebs, C. J. (1993) Influence of female relatedness on the demography of Townsend's vole populations in spring. *Journal of Animal Ecology,* **62,** 536-550.

Lambin, X. & Yoccoz, N. G. (1998) The impact of population kin-structure on nestling survival in Townsend's voles, Microtus townsendii. *Journal of Animal Ecology,* **67,** 1-16.

Lande, R. (1979) Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution,* **33,** 402-416.

Lande, R. (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution,* **34,** 292-305.

Lande, R. (1982) A quantitative genetic theory of life-history evolution. *Ecology,* **63,** 607-615.

Lande, R. & Arnold, S. J. (1983) The measurement of selection on correlated characters. *Evolution,* **37,** 1210-1226.

Langvatn, R., Mysterud, A., Stenseth, N. C. & Yoccoz, N. G. (2004) Timing and sychrony of ovulation in red deer constrained by short northern summers. *The American Naturalist,* **163,** 763-772.

Le Boeuf, B. J. (1974) Male-male competition and reproductive success in elephant seals. *American Zoologist,* **14,** 163-176.

Le Boeuf, B. J. & Mesnick, S. (1991) Sexual behavior of male northern elephant seals I. Lethal injuries to adult females. *Behaviour,* **116,** 143-162.

Legendre, P. (1993) Spatial autocorrelation - trouble or new paradigm. *Ecology,* **74,** 1659-1673.

Lehmann, L. & Perrin, N. (2003) Inbreeding avoidance through kin recognition: choosy females boost male dispersal. *The American Naturalist,* **162,** 638-652.

Lehtonen, T. K., Wong, B. B. M. & Lindstrøm, K. (2010) Fluctuating mate preferences in a marine fish. *Biology Letters,* **6,** 21-23.

Lincoln, G. A. (1992) Biology of antlers. *Journal of Zoology,* **226,** 517-528.

Little, T. J., Perutz, M., Palmer, M., Crossan, C. & Braithwaite, V. A. (2008) Male three-spined sticklebacks Gasterosteus aculeatus make antibiotic nests: a novel form of parental protection? *Journal of Fish Biology,* **73,** 2380-2389.

Lovari, S., Bartolommei, P., Meschi, F. & Pezzo, F. (2008) Going out to mate: excursion behaviour of female roe deer. *Ethology,* **114,** 886-896.

Lush, J. L. (1945) *Animal Breeding Plans,* Iowa State University Press, Ames.

Lynch, M. & Walsh, B. (1998) *Genetics and analysis of quantitative traits,* Sinauer Associates, Inc.

MacColl, A. D. C. & Hatchwell, B. J. (2003) Heritability of parental effort in a passerine bird. *Evolution,* **57,** 2191-2195.

Magnussen, S. (1993) Bias in genetic variance estimates due to spatial autocorrelation. *Theoretical and Applied Genetics,* **86,** 349-355.

Malo, A. F., Roldan, E. R. S., Garde, J., Soler, A. J. & Gomendio, M. (2005) Antlers honestly advertise sperm production and quality. *Proceedings of the Royal Society B-Biological Sciences,* **272,** 149-157.

Manson, J. H. (1994) Male-aggression - a cost of female mate choice in Cayo-Santiago Rhesus Macaques. *Animal Behaviour,* **48,** 473-475.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology,* **7,** 639-655.

Marshall, V. T., Humfeld, S. C. & Bee, M. A. (2003) Plasticity of aggressive signalling and its evolution in male spring peepers, Pseudacris crucifer. *Animal Behaviour,* **65,** 1223-1234.

Martin, J. G. A., Nussey, D. H., Wilson, A. J. & Réale, D. (2011) Measuring individual differences in reaction norms in field and experimental studies: a power analysis of random regression models. *Methods in Ecology and Evolution,* early view.

Mays, J. H. L. & Hill, G. E. (2004) Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution,* **19,** 554-559.

McComb, K. & Clutton-Brock, T. (1994) Is Mate Choice Copying or Aggregation Responsible for Skewed Distributions of Females on Leks? *Proceedings of the Royal Society B-Biological Sciences,* **255,** 13-19.

McComb, K. E. (1991) Female choice for high roaring rates in red deer, Cervus-Elaphus. *Animal Behaviour,* **41,** 79-88.

McEachern, M. B., McElreath, R. L., Van Vuren, D. H. & Eadie, J. M. (2009) Another genetically promiscuous 'polygynous' mammal: mating system variation in Neotoma fuscipes. *Animal Behaviour,* **77,** 449-455.

McElligott, A. G., Birrer, M. & Vannoni, E. (2006) Retraction of the mobile descended larynx during groaning enables fallow bucks (Dama dama) to lower their formant frequencies. *Journal of Zoology,* **270,** 340-345.

McElligott, A. G. & Hayden, T. J. (1999) Context-related vocalization rates of fallow bucks, Dama dama. *Animal Behaviour,* **58,** 1095-1104.

McLain, D. (1992) Population density and the intensity of sexual selection on body length in spatially or temporally restricted natural populations of a seed bug. *Behavioral Ecology and Sociobiology,* **30,** 347-356.

McLain, D. K., Burnette, L. B. & Deeds, D. A. (1993) Within season variation in the intensity of sexual selection on body size in the bug Margus obscurator (Hemiptera Coreidae). *Ethology, ecology and evolution,* **5,** 75-86.

McLoughlin, P. D., Boyce, M. S., Coulson, T. & Clutton-Brock, T. (2006) Lifetime reproductive success and density-dependent, multi-variable resource selection. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 1449-1454.

McNab, B. K. (1963) Bioenergetics and determination of home range size. *The American Naturalist,* **97,** 133.

Mendoza-Cuenca, L. & Macías-Ordóñez, R. (2010) Female asynchrony may drive disruptive sexual selection on male mating phenotypes in a Heliconius butterfly. *Behavioral Ecology,* **21,** 144-152.

Merilä, J., Kruuk, L. E. B. & Sheldon, B. C. (2001b) Natural selection on the genetical component of variance in body condition in a wild bird population. *Journal of Evolutionary Biology,* **14,** 918-929.

Merilä, J. & Sheldon, B. C. (2000) Lifetime Reproductive Success and Heritability in Nature. *The American Naturalist,* **155,** 301-310.

Merilä, J., Sheldon, B. C. & Kruuk, L. E. B. (2001a) Explaining stasis: microevolutionary studies in natural populations. *Genetica,* **112,** 199-222.

Mills, S. C., Alatalo, R. V., Koskela, E., Mappes, J., Mappes, T. & Oksanen, T. A. (2007b) Signal reliability compromised by genotype-by-environment interaction and potential mechanisms for its preservation. *Evolution,* **61,** 1748-1757.

Mills, S. C., Grapputo, A., Koskela, E. & Mappes, T. (2007a) Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proceedings of the Royal Society B: Biological Sciences,* **274,** 143-150.

Milner, R. N. C., Detto, T., Jennions, M. D. & Backwell, P. R. Y. (2010) Experimental evidence for a seasonal shift in the strength of a female mating preference. *Behavioral Ecology,* **21,** 311-316.

Mitani, J. C. & Brandt, K. L. (1994) Social factors influence the acoustic variability in the long-distance calls of male chimpanzees. *Ethology,* **96,** 233-252.

Moller, A. (1989) Viability costs of male tail ornaments in a swallow. *Nature,* **339,** 132-135.

Møller, A. P. & Szép, T. (2005) Rapid evolutionary change in a secondary sexual character linked to climatic change. *Journal of Evolutionary Biology,* **18,** 481-495.

Moore, A. J. (1990a) The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection and intersexual selection. *Evolution,* **44,** 315-331.

Moore, A. J. (1990b) The inheritance of social-dominance, mating-behavior and attractiveness to mates in male Nauphoeta-Cinerea. *Animal Behaviour,* **39,** 388-397.

Moore, Allen J., Haynes, Kenneth F., Preziosi, Richard F. & Moore, Patricia J. (2002) The evolution of interacting phenotypes: genetics and evolution of social dominance. *The American Naturalist,* **160,** S186-S197.

Moore, A. J. & Moore, P. J. (1999) Balancing sexual selection through opposing mate choice and male competition. *Proceedings of the Royal Society B-Biological Sciences,* **266,** 711-716.

Morisita, M. (1962) *I*σ-Index, a measure of dispersion of individuals. *Researches on Population Ecology,* **4,** 1-7.

Moyes, K. (2007) Demographic consequences of individual variation. University of Kent.

Moyes, K., Nussey, D. H., Clements, M. N., Guinness, F. E., Morris, A., Morris, S., Pemberton, J. M., Kruuk, L. E. B. & Clutton-Brock, T. H. (2010) Advancing breeding phenology in response to environmental change in a wild red deer population. *Global Change Biology***,** early view.

Muñoz, A., Aparicio, J. M. & Bonal, R. (2008) Male barn swallows use different resource allocation rules to produce ornamental tail feathers. *Behavioral Ecology,* **19,** 404-409.

Mysterud, A., Bonenfant, C., Loe, L. E., Langvatn, R., Yoccoz, N. G. & Stenseth, N. C. (2008) The timing of male reproductive effort relative to female ovulation in a capital breeder. *Journal of Animal Ecology,* **77,** 469-477.

Mysterud, A., Holand, Ø., Røed, K. H., Gjøstein, H., Kumpula, J. & Nieminen, M. (2003) Effects of age, density and sex ratio on reproductive effort in male reindeer (Rangifer tarandus). *Journal of Zoology,* **261,** 341-344.

Neff, B. D. & Pitcher, T. E. (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology,* **14,** 19-38.

Neff, B. D. & Pitcher, T. E. (2008) Mate choice for non-additive genetic benefits: A resolution to the lek paradox. *Journal of Theoretical Biology,* **254,** 147-155.

Neff, B. D., Repka, J. & Gross, M. R. (2001) A Bayesian framework for parentage analysis: the value of genetic and other biological data. *Theoretical Population Biology,* **59,** 315-331.

Nielsen, R., Mattila, D. K., Clapham, P. J. & Palsboll, P. J. (2001) Statistical approaches to paternity analysis in natural populations and applications to the north atlantic humpback whale. *Genetics,* **157,** 1673-1682.

Nussey, D. H., Kruuk, L.E.B., Morris, A., Clements, M.N., Pemberton, J.M. & Clutton-Brock, T.H. (2009) Inter- and intrasexual variation in aging patterns across reproductive triats in a wild red deer population. *The American Naturalist,* **174,** 342-357.

Nussey, D. H., Clutton-Brock, T. H., Albon, S. D., Pemberton, J. & Kruuk, L. E. B. (2005a) Constraints on plastic responses to climate variation in red deer. *Biology Letters,* **1,** 457-460.

Nussey, D. H., Coltman, D. W., Coulson, T., Kruuk, L. E. B., Donald, A., Morris, S. J., Clutton-Brock, T. H. & Pemberton, J. (2005b) Rapidly declining fine-scale spatial genetic structure in female red deer. *Molecular Ecology,* **14,** 3395-3405.

Nussey, D. H., Pemberton, J., Donald, A. & Kruuk, L. E. B. (2006) Genetic consequences of human management in an introduced island population of red deer (Cervus elaphus). *Heredity,* **97,** 56-65.

Nussey, D. H., Postma, E., Gienapp, P. & Visser, M. E. (2005c) Selection on heritable phenotypic plasticity in a wild bird population. *Science,* **310,** 304-306.

Nussey, D. H., Wilson, A. J. & Brommer, J. E. (2007) The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology,* **20,** 831-844.

Olvido, A. E. & Mousseau, T. A. (1995) Effect of rearing environment on calling-song plasticity in the striped ground cricket. *Evolution,* **49,** 1271-1277.

Panhuis, T. M. & Wilkinson, G. S. (1999) Exaggerated male eye span influences contest outcome in stalk-eyed flies (Diopsidae). *Behavioral Ecology and Sociobiology,* **46,** 221-227.

Parker, G. A. (1979) Sexual selection and sexual conflict. *Sexual selection and reproductive competition in insects* (eds M. S. Blum & N. A. Blum), pp. 123-166. Academic Press, New York.

Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. B. & Soutar, D. M. (2009) *Genstat for Windows (12th Edition) Introduction,* VSN International, Hemel Hempstead.

Pemberton, J. M. (2008) Wild pedigrees: the way forward. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 613-621.

Pemberton, J. M. (2010) Evolution of quantitative traits in the wild: mind the ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **365,** 2431-2438.

Pemberton, J. M., Albon, S. D., Guinness, F. E., Clutton-Brock, T. H. & Dover, G. A. (1992) Behavioral estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. *Behavioral Ecology,* **3,** 66-75.

Pérez-Espona, S., Pérez-Barbería, F. J., McLeod, J. E., Jiggins, C. D., Gordon, I. J. & Pemberton, J. M. (2008) Landscape features affect gene flow of Scottish Highland red deer (Cervus elaphus). *Molecular Ecology,* **17,** 981-996.

Perlut, N. G., Freeman-Gallant, C. R., Strong, A. M., Donovan, T. M., Kilpatrick, C. W. & Zalik, N. J. (2008) Agricultural management affects evolutionary processes in a migratory songbird. *Molecular Ecology,* **17,** 1248-1255.

Petersson, E., Jarvi, T., Olsen, H., Mayer, I. & Hedenskog, M. (1999) Male-male competition and female choice in brown trout. *Animal Behaviour,* **57,** 777-783.

Petrie, M., Krupa, A. & Burke, T. (1999) Peacocks lek with relatives even in the absence of social and environmental cues. *Nature,* **401,** 155-157.

Pfister, C. A. (1998) Patterns of variance in stage-structured populations: Evolutionary predictions and ecological implications. *Proceedings of the National Academy of Sciences of the United States of America,* **95,** 213-218.

Piertney, S. B., MacColl, A. D. C., Lambin, X., Moss, R. & Dallas, J. F. (1999) Spatial distribution of genetic relatedness in a moorland population of red grouse (Lagopus lagopus scoticus). *Biological Journal of the Linnean Society,* **68,** 317-331.

Pigliucci, M. (2005) Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution,* **20,** 481-486.

Pinheiro, J. C. & Bates, D. M. (2000) *Mixed effects models in S and S-Plus,* Springer-Verlag, New York.

Pomeroy, P. P., Twiss, S. D. & Redman, P. (2000) Philopatry, Site Fidelity and Local Kin Associations within Grey Seal Breeding Colonies. *Ethology,* **106,** 899-919.

Poole, J. H. (1989) Mate guarding, reproductive success and female choice in African elephants. *Animal Behaviour,* **37,** 842-849.

Post, E. & Forchhammer, M. C. (2008) Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **363,** 2367-2373.

Powell, R. A. (2000) Animal home ranges and territories and home range estimators. *Research Techniques in Animal Ecology Controversies and Consequences* (eds L. Boitani & T. K. Fuller). Columbia University Press, New York.

Preston, B. T., Stevenson, I. R., Pemberton, J. M., Coltman, D. W. & Wilson, K. (2003) Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. *Proceedings of the Royal Society B: Biological Sciences,* **270,** 633-640.

Preston, B. T., Stevenson, I. R., Pemberton, J. M. & Wilson, K. (2001) Dominant rams lose out by sperm depletion - A waning success in siring counters a ram's high score in competition for ewes. *Nature,* **409,** 681-682.

Price, T. D. (1984) The Evolution of Sexual Size Dimorphism in Darwin's Finches. *The American Naturalist,* **123,** 500-518.

Punzalan, D., Rodd, H. & Rowe, L. (2010) Temporally variable multivariated sexual selection on sexually dimorphic traits in a wild insect population. *The American Naturalist,* **175,** 401-414.

Pusey, A. & Wolf, M. (1996) Inbreeding avoidance in animals. *Trends in Ecology & Evolution,* **11,** 201-206.

Pusey, A. E. (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology & Evolution,* **2,** 295-299.

Puts, D. A., Gaulin, S. J. C. & Verdolini, K. (2006) Dominance and the evolution of sexual dimorphism in human voice pitch. *Evolution and Human Behavior,* **27,** 283-296.

Puurtinen, M., Ketola, T. & Kotiaho, J. S. (2009) The good-genes and compatible-genes benefits of mate choice. *The American Naturalist,* **174,** 741-752.

Qvarnström, A. & Forsgren, E. (1998) Should females prefer dominant males? *Trends in Ecology & Evolution,* **13,** 498-501.

Qvarnstrom, A., Part, T. & Sheldon, B. C. (2000) Adaptive plasticity in mate preference linked to differences in reproductive effort. *Nature,* **405,** 344-347.

Réale, D., Dingemanse, N. J., Kazem, A. J. N. & Wright, J. (2010) Evolutionary and ecological approaches to the study of personality. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **365,** 3937-3946.

Réale, D. & Festa-Bianchet, M. (2000) Quantitative genetics of life-history traits in a long-lived wild mammal. *Heredity,* **85,** 593-603.

Réale, D., McAdam, A. G., Boutin, S. & Berteaux, D. (2003) Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **270,** 591-596.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. (2007) Integrating animal temperament within ecology and evolution. *Biological Reviews,* **82,** 291-318.

Reby, D., Cargnelutti, B., Joachim, J. & Aulagnier, S. (1999) Spectral acoustic structure of barking in roe deer (Capreolus capreolus). Sex-, age- and individual-related variations. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie,* **322,** 271-279.

Reby, D., Charlton, B. D., Locatelli, Y. & McComb, K. (2010) Oestrous red deer hinds prefer male roars with higher fundamental frequencies. *Proceedings of the Royal Society B: Biological Sciences*.

Reby, D., Hewison, M., Izquierdo, M. & Pepin, D. (2001) Red deer (Cervus elaphus) hinds discriminate between the roars of their current harem-holder stag and those of neighbouring stags. *Ethology,* **107,** 951-959.

Reby, D. & McComb, K. (2003a) Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Animal Behaviour,* **65,** 519-530.

Reby, D. & McComb, K. (2003b) Vocal communication and reproduction in deer. *Advances in the Study of Behavior, Vol 33*, pp. 231-264.

Reichard, M., Jurajda, P. & Smith, C. (2004b) Male-male interference competition decreases spawning rate in the European bitterling (Rhodeus sericeus). *Behavioral Ecology and Sociobiology,* **56,** 34-41.

Reichard, M., Smith, C. & Bryja, J. (2008) Seasonal change in the opportunity for sexual selection. *Molecular Ecology,* **17,** 642-651.

Reichard, M., Smith, C. & Jordan, W. C. (2004a) Genetic evidence reveals density-dependent mediated success of alternative mating behaviours in the European bitterling (Rhodeus sericeus). *Molecular Ecology,* **13,** 1569-1578.

Reid, J., Arcese, P., Cassidy, A. E. V., Marr, A., Smith, J. M. & Keller, L. (2005) Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in song sparrows (Melospiza melodia). *Proceedings of the Royal Society B: Biological Sciences,* **272,** 481-487.

Reid, J. M. (2007) Secondary sexual ornamentation and non-additive genetic benefits of female mate choice. *Proceedings of the Royal Society B: Biological Sciences,* **274,** 1395-402.

Reid, J. M., Arcese, P., Sardell, R. J. & Keller, L. F. (2011) Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. *The American Naturalist,* **177,** 177-187.

Reid, J. M. & Keller, L. F. (2010) Correlated inbreeding among relatives: occurence, magnitude, and implications. *Evolution,* **64,** 973-985.

Relyea, R. A. & Demarais, S. (1994) Activity of desert mule deer during the breeding-season. *Journal of Mammalogy,* **75,** 940-949.

Rhodes, J. R., McAlpine, C. A., Lunney, D. & Possingham, H. P. (2005) A spatially explicit habitat selection model incorporating home range behavior. *Ecology,* **86,** 1199-1205.

Rhodes, M., Wardell-Johnson, G. W., Rhodes, M. P. & Raymond, B. E. N. (2006) Applying network analysis to the conservation of habitat trees in urban environments: a case study from Brisbane, Australia. *Conservation Biology,* **20,** 861-870.

Richard, E., Morellet, N., Cargnelutti, B., Angibault, J. M., Vanpe, C. & Hewison, A. J. M. (2008) Ranging behaviour and excursions of female roe deer during the rut. *Behavioural Processes,* **79,** 28-35.

Riede, T. & Fitch, T. (1999) Vocal tract length and acoustics of vocalization in the domestic dog (Canis familiaris). *J Exp Biol,* **202,** 2859-2867.

Rioux-Paquette, E., Festa-Bianchet, M. & Coltman, D. W. (2010) No inbreeding avoidance in an isolated population of bighorn sheep. *Animal Behaviour,* **80,** 865-871.

Rivera-Gutierrez, H. F., Pinxten, R. & Eens, M. (2011) Songs differing in consistency elicit differential aggressive response in territorial birds. *Biology Letters*.

Roberts, Craig, S., Gosling & Morris, L. (2003) Genetic similarity and quality interact in mate choice decisions by female mice. *Nature Genetics,* **35,** 4.

Robinson, M. R., Wilson, A. J., Pilkington, J. G., Clutton-Brock, T. H., Pemberton, J. M. & Kruuk, L. E. B. (2009) The impact of environmental heterogeneity on genetic architecture in a wild population of Soay Sheep. *Genetics,* **181,** 1639-1648. Rodriguez, R. L. & Greenfield, M. D. (2003) Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution,* **57,** 1304-1313.

Rodríguez-Muñoz , R., Bretman, A., Slate, J., Walling, C. A. & Tregenza, T. (2010) Natural and Sexual Selection in a Wild Insect Population. *Science,* **328,** 1269-1272.

Roff, D. A. & Rausher, M. (2007) A centennial celebration for quantitative genetics. *Evolution,* **61,** 1017-1032.

Rossiter, S. J., Ransome, R. D., Faulkes, C. G., Le Comber, S. C. & Jones, G. (2005) Mate fidelity and intra-lineage polygyny in greater horseshoe bats. *Nature,* **437,** 408-411.

Rowe, L. & Houle, D. (1996) The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **263,** 1415-1421.

Rukstalis, M., Fite, J. E. & French, J. A. (2003) Social Change Affects Vocal Structure in a Callitrichid Primate (Callithrix kuhlii). *Ethology,* **109,** 327-340.

San Jose, C. & Lovari, S. (1998) Ranging movements of female roe deer: Do home-loving does roam to mate? *Ethology,* **104,** 721-728.

Sanvito, S., Galimberti, F. & Miller, E. H. (2007) Vocal signalling of male southern elephant seals is honest but imprecise. *Animal Behaviour,* **73,** 287-299.

Say, L., Pontier, D. & Natoli, E. (2001) Influence of oestrus synchronization on male reproductive success in the domestic cat (Felis catus L.). *Proceedings of the Royal Society B: Biological Sciences,* **268,** 1049-1053.

Schielzeth, H. & Forstmeier, W. (2009) Conclusions beyond support: overconfident estimates in mixed models. *Behavioral Ecology,* **20,** 416-420.

Schuett, W., Tregenza, T. & Dall, S. R. X. (2010) Sexual selection and animal personality. *Biological Reviews,* **85,** 217-246.

Seaman, D. E. & Powell, R. A. (1996) An evaluation of the accuracy of kernel density estimators for home range analysis. *Ecology,* **77,** 2075-2085.

Sheldon, B. C. & Ellegren, H. (1999) Sexual selection resulting from extrapair paternity in collared flycatchers. *Animal Behaviour,* **57,** 285-298.

Shorey, L., Piertney, S., Stone, J. & Hoglund, J. (2000) Fine-scale genetic structuring on Manacus manacus leks. *Nature,* **408,** 352-353.

Shuster, S. M. (2009) Sexual selection and mating systems. *Proceedings of the National Academy of Sciences,* **106,** 10009-10016.

Shuster, S. M. & Wade, M. J. (2003) *Mating systems and strategies,* Princeton University Press.

Sih, A., Bell, A. & Johnson, J. C. (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution,* **19,** 372-378.

Silk, J. B., Alberts, S. C. & Altmann, J. (2003) Social bonds of female baboons enhance infant survival. *Science,* **302,** 1231-1234.

Simmons, L. W. (1991) Female choice and the relatedness of mates in the field cricket, Gryllus-Bimaculatus. *Animal Behaviour,* **41,** 493-501.

Sinervo, B. & Lively, C. M. (1996) The rock-paper-scissors game and the evolution of alternative male strategies. *Nature,* **380,** 240-243.

Singer, F. J. & Zeigenfuss, L. C. (2002) Influence of trophy hunting and horn size on mating behaviour and survivorship of mountain sheep. *Journal of Mammalogy,* **83,** 682-698.

Slate, J., Kruuk, L. E. B., Marshall, T. C., Pemberton, J. M. & Clutton-Brock, T. H. (2000) Inbreeding depression influences lifetime breeding success in a wild population of red deer (Cervus elaphus). *Proceedings of the Royal Society of London B: Biological Sciences,* **267,** 1657-1662.

Slate, J. & Pemberton, J. M. (2002) Comparing molecular measures for detecting inbreeding depression. *Journal of Evolutionary Biology,* **15,** 20-31.

Slate, J., Visscher, P. M., MacGregor, S., Stevens, D., Tate, M. L. & Pemberton, J. M. (2002) A genome scan for quantitative trait loci in a wild population of red deer (Cervus elaphus). *Genetics,* **162,** 1863-1873.

Smiseth, P. T., Wright, J. & Kölliker, M. (2008) Parent-offspring conflict and co-adaptation: behavioural ecology meets quantitative genetics. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 1823-1830.

Smith, R. H. (1979) On selection for inbreeding in polygynous animals. *Heredity,* **43,** 205-211.

Sparks, T. H. & Menzel, A. (2002) Observed changes in seasons: an overview. *International Journal of Climatology,* **22,** 1715-1725.

Sparreboom, M. (1996). Sexual Interference in the Sword-tailed Newt, Cynops ensicauda popei (Amphibia: Salamandridae). *Ethology* **10,**:672-685.

Stevenson, I. R. & Bancroft, D. R. (1995) Fluctuating Trade-Offs Favour Precocial Maturity in Male Soay Sheep. *Proceedings: Biological Sciences,* **262,** 267-275.

Stoner, G. & Breden, F. (1988) Phenotypic differentiation in female preference related to geographic variation in male predation risk in the Trinidad guppy (Poecilia reticulata). *Behavioral Ecology and Sociobiology,* **22,** 285-291.

Stopher, K. V., Pemberton, J. M., Clutton-Brock, T. H. & Coulson, T. (2008) Individual differences, density dependence and offspring birth traits in a population of red deer. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 2137-2145.

Storz, J. F. (1999) Genetic Consequences of Mammalian Social Structure. *Journal of Mammalogy,* **80,** 553-569.

Sugg, D. W., Chesser, R. K., Stephen Dobson, F. & Hoogland, J. L. (1996) Population genetics meets behavioral ecology. *Trends in Ecology & Evolution,* **11,** 338-342.

Sundaresan, S. R., Fischhoff, I. R. & Rubenstein, D. (2007) Male harassment influences female movements and associations in Grevy's zebra (Equus grevyi). *Behavioral Ecology,* **18,** 860-865.

Szulkin, M., Zelazowski, P., Nicholson, G. & Sheldon, B.C. (2009) Inbreeding avoidance under different null models of random mating in the great tit. *Journal of Animal Ecology,* **78,** 778-788.

Taylor, A. M., Reby, D. & McComb, K. (2009) Context-Related Variation in the Vocal Growling Behaviour of the Domestic Dog (Canis familiaris). *Ethology,* **115,** 905-915.

Taylor, P. D. & Williams, G. C. (1982) The Lek Paradox Is Not Resolved. *Theoretical Population Biology,* **22,** 392-409.

Team, R. D. C. (2008) R: a language and evironment for statistical computing. R foundation for Statistical Computing, Vienna.

Thackeray, S. J., Sparks, T. H., Frederiksen, M., Burthe, S., Bacon, P. J., Bell, J. R., Botham, M. S., Brereton, T. M., Bright, P. W., Carvalho, L., Clutton-Brock, T. H., Dawson, A., Edwards, M., Elliott, J. M., Harrington, R., Johns, D., Jones, I. D., Jones, J. T., Leech, D. I., Roy, D. B., Scott, W. A., Smith, M., Smithers, R. J., Winfield, I. J. & Wanless, S. (2010) Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology,* **16,** 3304-3313.

Thouless, C. R. (1990) Feeding competition between grazing red deer hinds. *Animal Behaviour,* **40,** 105-111.

Titze, I. R. (1994) *Principles of voice production,* Englewood Cliffs NJ: Prentice Halls.

Tomkins, J. L., Radwan, J., Kotiaho, J. S. & Tregenza, T. (2004) Genic capture and resolving the lek paradox. *Trends in Ecology & Evolution,* **19,** 323-328.

Trail, P. W. (1985) Courtship disruption modifies mate choice in a lek-breeding bird. *Science,* **227,** 778-780.

Travis, S. E., Slobodchikoff, C. N. & Keim, P. (1995) Ecological and demographic effects on intraspecific variation in the social system of Prairie Dogs. *Ecology,* **76,** 1794-1803.

Trivers (1972). *Sexual Selection and the Descent of Man, 1871-1971* (ed B. Campbell), pp. 136-179. Aldine-Atherton, Chicago.

Tufto, J., Andersen, R. & Linnell, J. (1996) Habitat use and ecological correlates of home range size in a small cervid: The roe deer. *Journal of Animal Ecology,* **65,** 715-724.

Turelli, M. & Barton, N. H. (2004) Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and G x E Interactions. *Genetics,* **166,** 1053-1079.

Twiss, S. D., Poland, V. F., Graves, J. A. & Pomeroy, P. P. (2006) Finding fathers: spatio-temporal analysis of paternity assignment in grey seals (Halichoerus grypus). *Molecular Ecology,* **15,** 1939-1953.

Twiss, S. D., Thomas, C., Poland, V., Graves, J. A. & Pomeroy, P. (2007) The impact of climatic variation on the opportunity for sexual selection. *Biology Letters,* **3,** 12-15.

Valcu, M. & Kempenaers, B. (2010) Spatial autocorrelation: an overlooked concept in behavioral ecology. *Behavioral Ecology,* **21,** 902-905.

Van der Jeugd, H. P. & McCleery, R. (2002) Effects of spatial autocorrelation, natal philopatry and phenotypic plasticity on the heritability of laying date. *Journal of Evolutionary Biology,* **15,** 380-387.

Van Winkle, W. (1975) Comparison of Several Probabilistic Home Range Models. *Journal of Wildlife Management,* **39,** 118-123.

Vannoni, E. & McElligott, A. G. (2009) Fallow bucks get hoarse: vocal fatigure as a possible signal to conspecifics. *Animal Behaviour,* **78,** 3-10.

Vannoni, E. M., A.G. (2008) Low Frequency Groans Indicate Larger and More Dominant Fallow Deer (Dama dama) Males. *PLoS ONE,* **3**.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D. & Van Tienderen, P. H. (1995) Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology & Evolution,* **10,** 212-217.

Visscher, P. M., Medland, S. E., Ferreira, M. A. R., Morley, K. I., Zhu, G., Cornes, B. K., Montgomery, G. W. & Martin, N. G. (2006) Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. *PLoS Genet,* **2,** e41.

Visser, M. E. (2008) Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 649-659.

Wade, M. J. & Shuster, S. M. (2004) Sexual selection: harem size and the variance in male reproductive success. *The American Naturalist*.

Walling, C. A., Pemberton, J. M., Hadfield, J. D. & Kruuk, L. E. B. (2010) Comparing parentage inference software: reanalysis of a red deer pedigree. *Molecular Ecology,* **19,** 1914-1928.

Wang, J. & Santure, A. W. (2009) Parentage and Sibship Inference From Multilocus Genotype Data Under Polygamy. *Genetics,* **181,** 1579-1594.

Ward, A. I. (2005) Expanding ranges of wild and feral deer in Great Britain. *Mammal Review,* **35,** 165-173.

Ward, P. I. (1998) A possible explanation for cryptic female choice in the yellow dung fly, Scathophaga stercoraria (L.). *Ethology,* **104,** 97-110.

Waser, P. M., Austad, S. N. & Keane, B. (1986) When should animals tolerate inbreeding? *The American Naturalist,* **128,** 529-537.

Weatherhead, P. J. (2005) Long-term decline in a red-winged blackbird population: ecological causes and sexual selection consequences. *Proceedings of the Royal Society B: Biological Sciences,* **272,** 2313-2317.

Wei, M. & van der Werf, J. H. (1993) Animal model estimation of additive and dominance variances in egg production traits of poultry. *J. Anim Sci.,* **71,** 57-65.

West, P. M. & Packer, C. (2002) Sexual selection, temperature, and the lion's mane. *Science,* **297,** 1339-1343.

West, S. A., Pen, I. Griffin, A.S. (2002) Cooperation and competition between relatives. *Science,* **296,** 72-75.

Wey, T., Blumstein, D. T., Shen, W. & Jordán, F. (2008) Social network analysis of animal behaviour: a promising tool for the study of sociality. *Animal Behaviour,* **75,** 333-344.

Whitehead, H. (2008) *Analyzing animal societies,* University of Chicago Press.

Whitehouse, M. E. A. (1997) Experience influences male-male contests in the spider Argyrodes antipodiana (Theridiidae: Araneae). *Animal Behaviour,* **53,** 913-923.

Wilham, R. L. (1972) The role of maternal effects in animal breeding. *Journal of Animal Science,* **28,** 74-81.

Williams, G. C. (1966) *Adaptation and Natural Selection: A Critique of Some Current Evolutionary Thought,* Princeton University Press, Princeton.

Williams, M. B. (1978) Sexual selection, adaptation, and ornamental traits: The advantage of seeming fitter. *Journal of Theoretical Biology,* **72,** 377-383.

Wilson, A. J., Coltman, D. W., Pemberton, J. M., Overall, A. D. J., Byrne, K. A. & Kruuk, L. E. B. (2005) Maternal genetic effects set the potential for evolution in a free-living vertebrate population. *Journal of Evolutionary Biology,* **18,** 405-414.

Wilson, A. J., Morrissey, M. B., Adams, M. J., Walling, C. A., Guinness, F. E., Pemberton, J. M., Clutton-Brock, T. H. & Kruuk, L. E. B. (2011) Indirect genetics effects and evolutionary constraint: an analysis of social dominance in red deer, Cervus elaphus. *Journal of Evolutionary Biology***,** in press.

Wilson, A. J., Pemberton, J. M., Pilkington, J. G., Coltman, D. W., Mifsud, D. V., Clutton-Brock, T. H. & Kruuk, L. E. B. (2006) Environmental Coupling of Selection and Heritability Limits Evolution. *PLoS Biol,* **4,** e216.

Wolf, J. B., Brodie, E. D., Cheverud, J. M., Moore, A. J. & Wade, M. J. (1998) Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution,* **13,** 64-69.

Wolff, J. O. (1998) Breeding Strategies, Mate Choice, and Reproductive Success in American Bison. *Oikos,* **83,** 529-544.

Wong, B. B. M. & Candolin, U. (2005) How is female mate choice affected by male competition? *Biological Reviews,* **80,** 559-571.

Worthington Wilmer, J., Overall, A. J., Pomeroy, P. P., Twiss, S. D. & Amos, W. (2000) Patterns of paternal relatedness in British grey seal colonies. *Molecular Ecology,* **9,** 283-292.

Worton, B. J. (1987) A Review of Models of Home Range for Animal Movement. *Ecological Modelling,* **38,** 277-298.

Worton, B. J. (1989) Kernel Methods for Estimating the Utilization Distribution in Home-Range Studies. *Ecology,* **70,** 164-168.

Wright, S. (1984) *Evolution and the genetics of populations,* Chicago Press, Chicago.

Appendix A

Examining the effect of using categorical pedigrees

The use of categorical pedigrees - in which paternities assigned with a specific confidence (in this study, over 80%) are taken as the 'true' pedigree, are potentially misleading as they do not incorporate the uncertainty around those paternity assignments.

The program "MasterBayes", which uses a Bayesian method of parentage assignment (Hadfield 2006), calculates many iterations of the potential pedigree, with the point estimate of the pedigree accepting those paternity assignments which appear in 80% of iterations. Instead of calculating a point estimate of the pedigree statistics in Chapter 5 from the categorical pedigree, by calculating pedigree statistics across many of the Bayesian iterations, an error can be attached to those pedigree statistics, thereby accounting for error in paternity assignments.

Methods

The key pedigree statistics calculated in Chapter 5- female re-mating frequency, intra-lineage polygyny, inbreeding coefficients and relatedness coefficients - were calculated for each of 1000 iterations of the MasterBayes pedigree, and average values calculated with standard deviations.

The aim of this analysis was to ask whether the discrepancies between the observed pedigree statistics and the simulation data (see main text) could be explained by error around the paternity assignments. Therefore, for each of the key pedigree statistics, we took the distribution of values from the simulation which most closely modelled that seen in the observed pedigree, and compared that to the distribution of values from the 1000 MasterBayes iterations (hereafter known as 'Observed with error' pedigrees). The distributions were compared using Wilcoxon tests (non-parametric tests were appropriate because of unequal variances).

It should be noted at this point that the 'Observed' pedigree, used in the main text, which uses categorical paternity assignments, is constructed using a combination of MasterBayes and the program "COLONY2" (see main text). Therefore, parameters describing the 'Observed' pedigree and 'Observed with Error' pedigrees are not directly comparable.

Results

Re-mating frequency of females

Using the 'Observed with Error' dataset, 27.19±0.61% of females were estimated to re-mate with the same male in multiple years. The values of this distribution were significantly greater than the 'Age Corrected' simulation (18.90±1.32, W=1000000, p<0.0001), indicating more re-mating was occurring more than expected under any simulated mating scenario even after accounting for pedigree error.

Intra-lineage polygyny

The ratio of unique females a male mated with to matrilines mated with was estimated at 0.807±0.005 for the 'Observed with Error' pedigrees. This was significantly lower than in the 'Age Corrected' simulation (W=46729, p<0.0001), indicating significantly more intra-lineage polygyny was occurring than in the simulated data.

Relatedness

The average relatedness coefficient in the "Observed with Error" dataset was 0.00566±0.00008. This was significantly higher than in the "Spatial 100m" simulation (0.00464±0.00020, W=1000000, p<0.0001). Therefore, pairs of individuals were still more related in this dataset than in the most closely fitting simulation when pedigree error was taken into account.

Inbreeding

Average inbreeding coefficients were calculated for the 'Observed with Error' pedigrees as 0.00233±0.00108. This was significantly higher than under the simulation scenario which produced the largest average inbreeding coefficients (Spatial 100m, W=851602, p<0.0001). There were significantly more non-zero coefficients in the Observed Error pedigrees (266.92±11.22) than in the Spatial 100m (W=990224, p<0.0001; note Spatial 100m does not have the largest number of non-zero coefficients of all the simulations, which occur in the "Temporal" simulation, but the number is similar, 206.21±21.63 versus 217.57±20.09, and so the comparison is made using Spatial 100m for consistency). However, there were not significantly more close inbreeding events in the 'Observed with Error' pedigree (25.57±1.80) than in the Spatial 100m simulation (26.43±4.74,

W=448365, p=1.00). Therefore, taking into account pedigree error, our conclusions surrounding inbreeding remain as presented in the main text: inbreeding is greater in the observed population than expected from any simulation, but due to an increase in total non-zero inbreeding coefficients, rather than close inbreeding events.

Appendix B

Variation in re-mating frequency and consequences for fitness

Although the consequences of females re-mating with the same male in multiple years on population genetic structure are not affected by the proximate causes of this behaviour, by understanding variation in the probability of re-mating, and the fitness consequences of such behaviour, we can understand whether it is adaptive, and how it might have evolved. By incorporating spatial and temporal information into the simulated pedigrees, we have demonstrated that some, but not all, of re-mating behaviour can be explained by site and temporal fidelity of rutting individuals. This represents a significant improvement on previous work in mammals, in which only anecdotal evidence only has so far been used to argue that all re-mating is unlikely to be explained by site fidelity or temporal fidelity (Rossiter *et al.* 2005, Amos *et al.* 1995). In avian systems, the importance of site fidelity in re-establishing monogamous pairs in sexually segregated species has received more detailed attention: a phylogenetic analysis of the Ciconiiform family of birds suggests that re-mating tendencies are directly related to the degree of site fidelity shown by pairs, and evolved only after it was enabled by the evolution of site fidelity (Cézilly *et al.* 2000).

However, if not all re-mating is explained by constraints or artefacts of the mating system, the evolution of re-mating requires that it results in fitness benefits to pairs which mate in consecutive seasons. In avian systems, the 'success-stay-failure-leave' theory has received much attention (Dubois and Cézilly 2002). Pairs are expected to remain together if they have successfully reared offspring and 'divorce' upon reproductive failure; however, although evidence generally supports this idea, the strength of the correlation between offspring success and re-mating tendency can vary with individual or pair-based factors such as the timing of reproductive failure, pair experience or disparity of timing of arrival in future breeding seasons (Dubois and Cézilly 2002). In the few examples of mammalian re-mating, the effect of offspring success on rates of future re-mating is unknown, as more generally is whether there are benefits to offspring fitness which drive the evolution of this behaviour. In grey seals, mate fidelity has been suggested to reduce pup pre-weaning mortality by reducing the rate of disturbance from aggression interactions between males (Amos 1995), but this is untested.

Here we examine the factors affecting the frequency with which male-female pairs of red deer mated in multiple years, and the fitness consequences of doing so.

Methods

Factors affecting frequency of re-mating

The factors affecting the frequency with which pairs re-mated were investigated using a Generalized Linear Mixed Model (GLMM). As the response variable we fitted a binary variable denoting whether the pair re-mated at each opportunity to do so following their first pairing *(i.e.* in any year following the initial pairing in which the female conceived and the male was known to be rutting); 1 denoted the pair re-mated, 0 that they did not (see Chapter 5 for more details). A binomial error structure was therefore used. Male and female identities were fitted as random effects, and the following as fixed effects:

Year: the year in which the pair had the opportunity to re-mate, fitted as a factor*;*

Offspring survival: whether the previous offspring born to the pair survived to the age of one (1 being survived, 0 died);

Female age and its quadratic term;

Male age and its quadratic term;

Female home range size: core home range size during the rut in hectares, calculated using daily census locations during the rut (available to within 100m accuracy, for more details see Chapter 6);

Female subdivision: the region of the study area of in which a female's average location fell during the rut: Kilmory Glen, Mid Glen, South Glen, Intermediate Area or Shamhan Insir (see Moyes 2007);

Male annual breeding success (ABS) : the annual breeding success of the male in the year in which the pair had the opportunity to re-mate, included to investigate whether pairs re-mate simply because the male is in general more likely to mate;

Female oestrus date: calculated by backdating from the birth date of the calf she subsequently produces by 235 days (Clutton-Brock *et al.* 1982);

Male rut start date: the first day of the rut on which the male held a harem.

Model simplification was done by stepwise removal of non-significant terms, tested using Wald statistics with a significance level of $\alpha=0.05$. Two way interactions were tested between terms which were significant as main effects.

Effects of re-mating on offspring fitness

We also tested whether pairs which mated in multiple years had offspring of higher fitness. Three response variables were analysed: birth date, birth weight and offspring survival to one year, in linear mixed effects models, with offspring sex, mother's age and its quadratic term, mother's reproductive status (see Chapter 6), birth year (as a factor) as explanatory variables, as well as whether the parents of the offspring mated with each other in more than one year over their reproductive lifespan (0 for no, 1 for yes). The identities of the parents were included as random effects. For survival to one, we fitted a generalised linear mixed effects model with binomial errors. Two way interactions between whether parents re-mated and all other fixed effects were included in the full model.

Results

Factors affecting frequency of re-mating

Pairs were significantly more likely to re-mate if the female came into oestrus earlier during the rut, and if the male began rutting earlier (see table A2.1, figure A2.1). Pairs were also more likely to re-mate if the male gained greater mating success in the year being considered, *i.e.* if in general the male was more likely to mate (see table A2.1, figure. A2.2); but independently of this, were less likely to re-mate when the male was older (see table A2.1, figure A2.3). Females were less likely to re-mate with a previous partner if they had a larger home range (see table A2.1, figure A2.4). Finally, the probability of pairs re-mating varied with year (see table A2.1, figure A2.5), but there was no consistent temporal trend (in the minimal model reported, changing year to a continuous variable rendered the term non-significant, $F_{1, 169.6}=0.54$, $p=0.46$).

Table A2.1: Results of a GLMM testing factors affecting whether pairs re-mated when they had the opportunity to do so. Estimates and standard errors are given, as well as F values, degrees of freedom and p values, for a Wald test, dropping individual terms from the full model. Effect sizes for years are not shown for brevity.

Term	Effect Size	S.E.	d.f.	F value	P value
Year	(Not shown)	(Not shown)	32,1043.1	7.70	<0.001
Male ABS	1.158	0.090	1,1110.7	165.83	<0.001
Female oestrus date	-0.341	0.026	1,1146.9	176.48	<0.001
Male rut start date	-0.369	0.033	1, 1120.9	121.87	<0.001
Female home range size	-0.025	0.005	1, 625.6	22.53	<0.001
Male age	-0.741	0.189	1, 374.6	15.22	<0.001

Figure A2.1: effect of female oestrus date and the date on which the male started rutting on the probability of pairs re-mating. Dotted lines indicate 95% confidence intervals around the regression line.

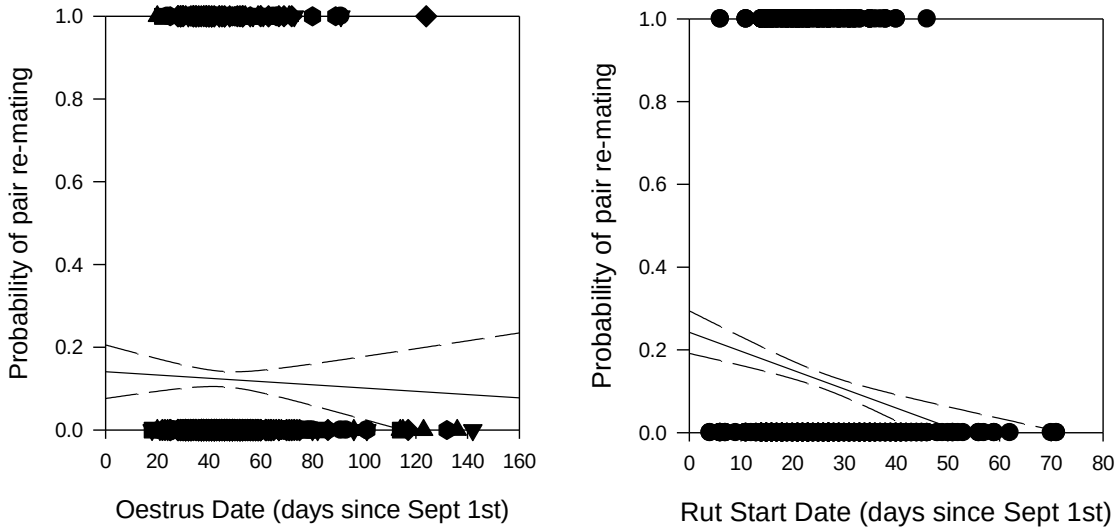


Figure A2.2: effect of male annual breeding success (ABS) on the probability of a pair re-mating. Dotted lines indicate 95% confidence intervals around the regression line.

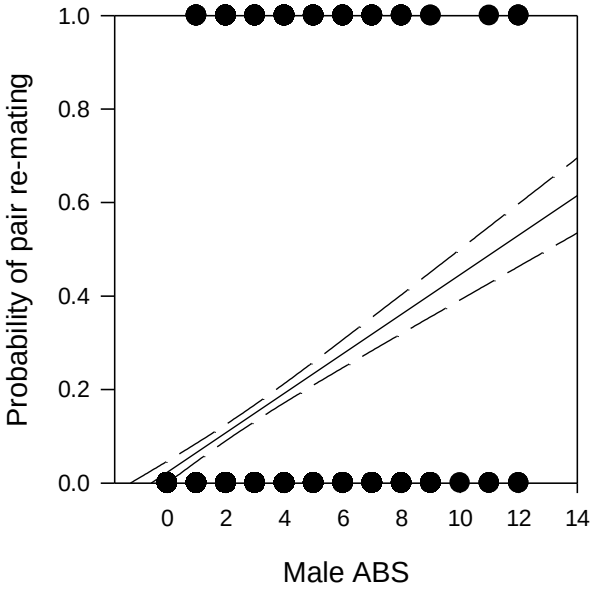


Figure A2.3: effect of male age at the time of potential re-mating on the probability of a pair re-mating.

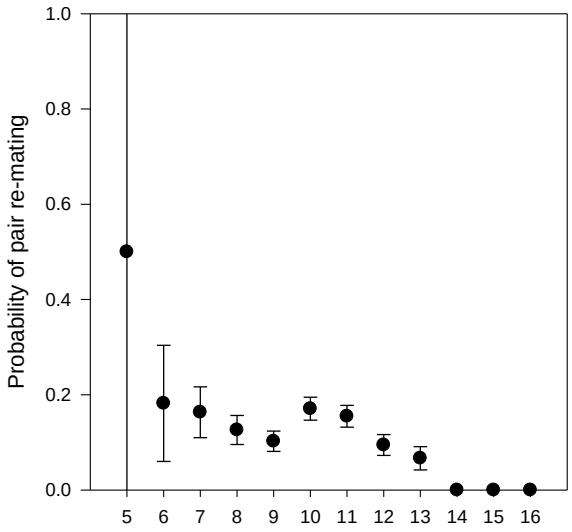


Figure A2.4: the effect of female home range size on the probability a pair will re-mate. Dotted lines indicate 95% confidence intervals around the regression line.

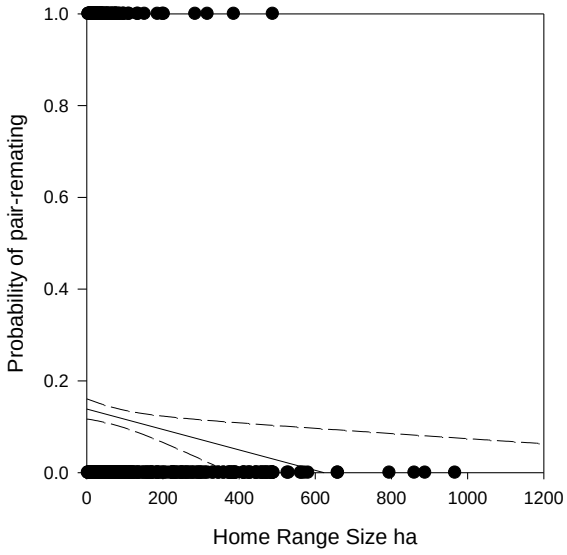
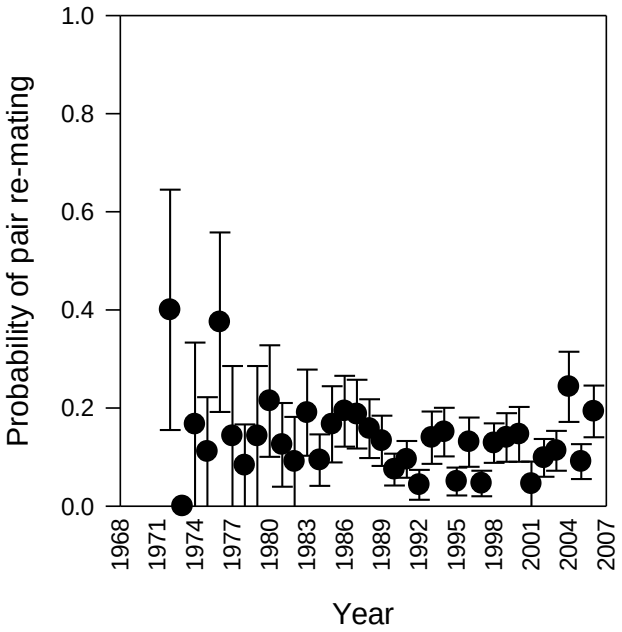


Figure A2.5: temporal changes in the probability of pairs re-mating.



Effects of re-mating on offspring fitness (table A2.2)

Offspring born to pairs which mated more than once were born around three days earlier (Est.=-2.925±0.893, $F_{1,1073.5}$=10.73, p=0.001); given the correlation between oestrus date and parturition date (Clements *et al.* 2010) this is an agreement with our findings that pairs were more likely to re-mate if the female was in oestrus earlier in the season. However, offspring born to pairs which mated in more than one year were not heavier (in maximal model containing no interactions, effect of parents re-mating on offspring birthweight: Est. =0.119±0.158, $F_{1,924.5}$=0.57, p=0.451) nor more likely to survive to 1 (in maximal model containing no interactions, effect of parents re-mating on offspring survival: Est.=-0.008±0.158, $F_{1,1070.6}$<0.01, p=0.960).

Table A2.2 Results of a LMM testing whether offspring born to pairs which re-mated had earlier birth dates. Estimates and standard errors are given, as well as F values, degrees of freedom and p values, for a Wald test, dropping individual terms from the full model.

Variable	Estimate	SE	F value	D.f	p
Intercept	162.000	0.990			
Maternal Reproductive Status			16.10	4,1070.1	<0.001
Maternal Reproductive Status (Naive)	0.571	1.100			
Maternal Reproductive Status (Summer Yeld)	-5.281	1.098			
Maternal Reproductive Status (True Yeld)	-4.775	0.889			
Maternal Reproductive Status (Winter Yeld)	2.165	1.134			
Birth Year	-0.415	0.086	23.15	1,242.4	<0.001
Pair re-mating (did re-mate)	-2.925	0.893	10.73	1,1073.5	0.001